

Helping out in Bhopal

It would be easy to draw the wrong conclusions from last week's harrowing events at Bhopal's Union Carbide plant.

BHOPAL in Mahdya Pradesh in central India is a long way from Danbury, Connecticut, where Union Carbide has its headquarters. In neat New England, everybody knows that it is difficult to win permission to build any kind of chemical plant within sight of human habitation and that, thereafter, zoning laws will ensure that the surroundings are not promptly occupied by people. From a vantage point in New England, it is not easy always to remember that, in India, people will instead gravitate towards shanty structures as close as possible to the gates of whatever factories there may be. How else to get to work?

So there is a sense in which the worst disaster so far caused by the chemical industry, at least since the destruction of the port of Halifax, Nova Scotia, in 1917, springs from the circumstance that the plant had been built in India. Two other Indian features of the tragedy stand out. First, the people working at the Bhopal plant seem to have been slow to appreciate the scale of the danger. Second, the anguish vividly carried round the world by the television cameras seems not to have matured into the anger, even hysteria, there would have been had the accident occurred on the edge of a European city — or in Connecticut. But to write off this sticism as a manifestation of Indian fatalism, or familiarity with death, would be greatly to demean the responsibility with which both the community of Bhopal and the authorities of the state and of the centre in New Delhi have behaved. That this should have been possible in the thick of an election campaign is doubly remarkable.

None of this guarantees that the affair will pass off without trouble for Union Carbide, or for the operators of other chemical plants in India and elsewhere in the developing world. The contingency lawyers will see to that. But as yet, everybody recognizes, it is too soon to apportion blame. That is what the inquiry now under way is for. Whatever the findings, however, one source of confusion stands out. Although the plant at Bhopal seems to be a carbon copy of one operated in West Virginia, Union Carbide owns only a narrow majority of the Indian operating company, with the remainder in the hands of Indian interests according to the formula by which India seeks to blunt the impact of overseas investment. So Union Carbide may be expected to shoulder only 51 per cent of whatever blame is found to lie at the door of the plant's owners and operators. Legal caution would therefore suggest that the company should await the outcome of the inquiry before deciding what to do, and that it should then await the outcome of whatever procedural niceties are required to determine its responsibilities, if any.

Urgency

The implied delay, perhaps for several years, would be a crying shame. The need for relief, and for arrangements to be set in hand to care for the victims of this tragedy, is urgent. Now is when the anxiety of those injured but not killed will be greatest, and when fears of an uncertain future will most prey on people's minds. Without prejudice, as the lawyers are forever saying, which in this case means that the US parent company may be strictly blameless, Union Carbide should make a quick start on the provision of the resources to ensure the medical services that will be needed for

decades ahead to care for the people exposed to methyl isocyanate ten days ago. If the company considers the task beyond its own resources (which is possible) it should cajole other Western corporations similarly at risk in India to share the burden. It is not too much to ask that the governments concerned should also help. The objective at this stage should simply be to put in place the facilities that Indian medicine will need to monitor the health of those who have been exposed, and to provide what treatment is possible. In this instance to act swiftly is more important than to act prudently.

But would not such an initiative imply an admission that blame applies? No, is the simple answer. The lawyers' *caveat* is full of meaning. And when was a proper sense of compassion ever blameworthy? But if India proudly insists that it wants not compassion (with all its neo-colonialist connotations), but only justice, this is nevertheless an occasion when compassion should be offered. Catastrophes on the scale of Bhopal are too great, and their implications too ramified, to be handled alone even by the wealthiest societies. And this is unlikely to be the last accident to occur with the dreadful character of what has happened at Bhopal.

Other superficial interpretations of what has happened also cry out for denial. Outside India, there has already been a wave of soothsaying to the effect that what happened at Bhopal shows that the "export" of advanced technology to developing countries is in some sense inappropriate. That is the true neo-colonialism. And as an opinion, it is clearly false. For one thing, India is far from being technologically innocent. Moreover, the indigenous chemical industry is as strong as any other Indian technology. But there is also a home-grown nuclear industry (whose plants have been managed successfully if, perhaps, less well than they might have been) not to mention a thriving indigenous space enterprise with its own satellites that will, in due course, also be launched indigenously. There is no sense in which a sophisticated plant for manufacturing pesticides is inherently out of place in India.

Interdependence

What is wrong with Indian technology is not the amount of it or even the state of development but that it is too thinly spread. For at least the past three decades, India has followed a policy of development on a broad front, on the grounds that only by attempting everything (and, in practice, doing a little of everything) would it be possible to shake off the colonial yoke. The result is that India does everything less well than superbly, and at least a little late. A better strategy would be to do some things so well that their products could be sold on the export markets, earning the foreign exchange with which to buy the products of the technologies left temporarily on one side. This is how other economic systems, most of those in Western Europe included, set out to match their capabilities against the complexity of modern technology. They have discovered that economic interdependence does not mean thralldom. Why should India be all that different? But the consequence, at Bhopal, would either have been that the methyl isocyanate plant would have been but one in



a larger and stronger indigenous industry or that it would not have been there at all, but perhaps in Connecticut.

The doctrine of self-reliance (as distinct from self-sufficiency) was always due to be an election issue. Now, after Bhopal, Mr Rajiv Gandhi will find himself also under pressure from the traditionalists who argue that India's error is to have aimed at something more ambitious than being a village economy. He can be relied upon to resist that claim, on the sure grounds that there is no other way than technology to meet the aspirations of the villagers. For has not the new agricultural technology, he can say, made them well-fed for the first time in history?

But on past form, Mr Gandhi will not turn his back on self-reliance. The two weeks remaining of the election campaign is not the time for changing tack, but afterwards there is the strongest case for looking again at this policy, seeking some way of concentrating resources on a field in which India could make a stronger mark.

Inappropriate

The other common false lesson drawn from what happened last week is that modern technology may as such be inappropriate, not merely in India but everywhere. People say "Look at Three Mile Island", intending to imply that the two accidents are similar and that the technologies that made them possible should ideally be avoided. In reality, nothing could or need be further from the truth. Plants for making pesticides of various kinds have operated safely and successfully during the past half-century; only some of them are risky, as these things go. (One of the ironies of the Bhopal accident is that the pesticide being manufactured, Sevin, is a substitute for DDT introduced in an attempt to avoid the risks of that organochlorine compound; the process for manufacturing the new pesticide has already done more damage than DDT.) But it is a good question whether large quantities of a dangerous chemical, particularly if it is an intermediate, should ever be stored as they were in India. Carrying these chemicals about the country (as is done in the United States) is also a needless risk. What needs to be learned from last week's accident in technically advanced societies is that the handling of novel chemical processes needs constant reassessment. □

Research charade

The UK research community should respond forcefully to the latest disappointments.

THE feckless mishandling of the British research enterprise by the British government has now been invested with an element of farce. So much is plain from last week's events, beginning with the government's attempts to limit the political damage done by Sir Keith Joseph's plan to make higher education more expensive for the student offspring of the well-to-do (see *Nature* 6 December, p. 483). Part of the plan to contain the damage was the accelerated publication of the document describing how £14 million of the funds thus "saved" would be used to make the British research councils less constrained. The chairman of the Advisory Board for the Research Councils (ABRC) dutifully stood up in public and said how valuable the extra money would be. But by the following day, Sir Keith Joseph had been forced by the House of Commons to go back on his proposals (see page 582) and by the Treasury to claw back £4 million of what he had been offering a few days earlier. The University Grants Committee, less quick off the mark, similarly had £6 million snatched back from the £10 million it had been offered for universities in crying need of new equipment, but without the added humiliation of having advertised its gratitude in public.

What this pantomime has done is to make a monkey of those who administer the British government's support of civil science, and of government policy on research in general. The common complaint by the research councils in recent years that they have not known where they stand from one year to the next has now been overtaken by the simple truth that they may not know from day to day. The simplest reason why all this is a nonsense is that if

the government was right, at the beginning of last week, to have taken the view that the need for extra funds for science had become so urgent as to justify the political risks of increasing higher education costs, it cannot have become, by the end of the week, proper for it to say that it would let the needs of research go hang.

Even those who believe the British government is right to insist that public expenditure should be tightly controlled will think it humbug that the government pretends that nowhere in its budget of £125,000 million is to be found £10 million that might be spared for the support of research. Indeed, the sum concerned is smaller than the errors that arise in estimating the various components of British public expenditure.

Ministers at other spending departments than the Department of Education and Science will now be quick to say that Joseph's muddle is his own affair, that he should have fought harder, some months ago, when next year's budget was being hammered out and that he should not now expect them to save him from the trouble he has brought down on himself. What, in all this, has happened to the doctrine of collective cabinet responsibility by which British governments set such store, especially when they need to make some recalcitrant minister toe the party line?

Several things should happen now, at least one of which should be a ritual resignation. Ordinarily, it would have been Sir Keith Joseph who would have packed his bags, but he and his government appear to have agreed that that may not be necessary. The members of ABRC and the heads of the research councils are differently placed. Most of them are, in any case, people with other and even better things to do, and cannot much profit from having been made to look like fools. To walk out at this stage would not bring the administration of science to a halt. To stay will be to let the government continue to behave as if the noise there has been about the condition of the British research enterprise is a ramp conceived by self-interested people eager to take for themselves and their cronies a larger share of the public pie, mostly for making their ivory towers more comfortable. Resignations after events such as those at last week can have no immediate effect. Their value is that they would show that people mean what they have been saying about the sombre condition of the research enterprise in Britain.

Initiative

For the rest, the British research community should resolve to seize the initiative for the management of its own affairs that will be there to seize in the period of embarrassment that is bound to follow. It will also be politically impossible to go back on the Prime Minister's promise five years ago that the science budget will be protected, which appears to mean that it will be indexed against inflation and then made to accommodate a variety of needs that were not apparent at the beginning. The best strategy now is that already being followed by two of the research councils (for science and the natural environment), which have embarked on redefinitions of their budgets so as to enhance their flexibility. The Agricultural and Food Research Council has already moved along that road. The need now is that this process should be radical, based on the assumption that the government, the research councils' paymaster, which is plainly indifferent to their present plight, will be equally indifferent to the ways in which they organize their affairs.

ABRC has a special role to play. It is the only committee on which the University Grants Committee and the research councils are both represented. It is also a means by which the British academic community as a whole could be consulted. To salvage the self-respect damaged by last week's events, ABRC should begin to throw its weight about more vigorously, even raising in public some of the questions about British research which are ordinarily considered taboo. Does it, for example, make sense that the British government should administer civil science by the account-book simply because that expenditure is identifiable and thus easily controlled, while allowing the vast apparatus of defence research and development to go its own way because it happens to be part of an even larger enterprise? □

Toxic gas

Pesticide plant leak wreaks disaster in India

New Delhi

THE leak of toxic gas from the Union Carbide pesticide plant in the central Indian town of Bhopal on 3 December has left at least 2,500 people dead and killed hundreds of cattle. The death toll is likely to increase, with thousands still in hospital three days after the disaster, which affected 200,000 people living within a 7-km radius of the plant. The cause of the leak is being investigated by chemicals experts with the help of a team flown in by the US multinational company. Meanwhile, the state government of Madhya Pradesh has arrested six workers, including the plant manager, ordered indefinite closure of the plant and started a judicial inquiry. The Central Bureau of Investigations is looking into the possibility of sabotage. It seems likely that Union Carbide India Limited

(UCIL), the wholly-owned US subsidiary, will be faced with large claims for damages.

The killer gas was methyl isocyanate (MIC), used in the plant at Bhopal as an intermediary in the manufacture of some pesticides, including Sevin, which is widely used. According to a UCIL spokesman, the leak occurred in one of three underground tanks storing MIC, which is liquid up to 38°C and extremely volatile. He said the vent caustic soda scrubbers provided for neutralizing accidental releases failed to cope with an excessive pressure build-up. The tank held 45 tons at the time of the leak. It is not known how much gas escaped from the plant which, flouting existing safety guidelines, is just 1 km from the railway station and 5 km from the centre of town. UCIL said that the leak was plugged 45 minutes after it was discovered, but by that time much of the town of Bhopal had been virtually turned into a gas chamber, and was a vivid demonstration of what chemical warfare would be like.

The UCIL plant, commissioned in 1977, employs 800 workers. Ironically, the Indian government only last year extended

the plant's licence for seven years after a promise from UCIL that it would secure from its parent US company the technology to handle "emergency situations like toxic gas release, sometimes accompanied with fire, endangering the safety of the community". There had been two earlier minor incidents in the Bhopal plant, one in 1981 when a leak from a phosgene storage tank killed several workers; in the latest incident, oddly enough, only one worker was injured.

In the nightmarish aftermath of Bhopal, the Indian incident is bound to become a factor in the forthcoming general election. The opposition is critical of India's policy of importing hazardous technology and pesticide licensing. The recent collaboration with Monsanto Corporation of the United States for manufacture of the herbicide butachlor, which is banned in the United States, is under attack. But pesticides made using indigenous technology in India are produced under lower safety conditions than those that existed at Bhopal, according to a spokesman for the Ministry of Chemicals. Six companies in India have been licensed to make MIC and several small pesticide manufacturers are transporting phosgene in bottles. Following the Bhopal tragedy, safety has been tightened at the state-owned Indian Petrochemicals Limited in Baroda which produces hydrocyanic acid, which is far more deadly than MIC.

K.S. Jayaraman

Ways of death

Washington

RELATIVELY little is known of the toxicological effects of methyl isocyanate, which is produced by reacting methylamine and phosgene and reacted with α -naphthol to produce the pesticide Sevin. Its strong primary irritant properties probably accounted for the majority of deaths: conversion to cyanide is not thought to occur at a significant rate *in vivo*. The US Occupational Safety and Health Administration limits exposure of workers to 0.02 parts per million over an 8-hour period. Tests on rats and on human volunteers indicate that the chemical's most immediate effects are upon the respiratory tract and the cornea; in rats, the cause of death was a massive build-up of fluid in the lungs. In humans, exposure to 2 parts per million caused nose and throat irritation, 21 parts per million a suffocating effect and severe irritation of the respiratory tract and eyes.

The cause of death after acute exposure would probably be either bronchospasm or what amounts to drowning in released body fluids. Failing this, death could result from the secondary effects of loss of blood oxygen and acidosis, or from congestive heart failure. According to Dr Trent Lewis of the National Institute of Occupational Safety and Health, survivors will probably suffer increased incidence of pulmonary fibrosis, emphysema and chronic bronchitis. The respiratory tract may become hyperreactive to a variety of irritants, and changes in lung architecture may increase susceptibility to infectious pneumonia. Necrotic lesions of the cornea may lead to permanent blindness or impaired vision.

Tim Beardsley

UK government review

More bad news for science

BRITISH government expenditure in basic science is due to fall by three per cent (at constant prices) over the next two years, according to the second *Annual Review of Government-Funded Research and Development* (HMSO, £7.50) published on Tuesday. Prepared by the department of the Chief Scientific Adviser at the Cabinet Office, Dr Robin Nicholson, this is a second and more accurate shot at determining the trends and distribution of government science spending.

The figures were prepared too late to include the recent goings-on at the Department of Education and Science (see p.582), but they do add some grist to the argument that science in Britain is threatened. Compared with 1983-84 expenditure, and in real terms, the Cabinet Office expected the Economic and Social Research Council budget to have fallen to 84 per cent of its original value by 1986-87; Agricultural and Food to 91 per cent; Medical to 94 per cent; Natural Environment to 98 per cent; and Science and Engineering to have risen to a princely 101 per cent.

In civil applied research ("expenditure to improve technology") things look only slightly better. The total government expenditure in this area will be £807 million in 1986-87, the Cabinet Office estimates, some 9 per cent greater in real terms than in

1983-84. But this represents a decrease from the current year (1984-85), which rides at 15 per cent above last year. The biggest projected declines to 1986-87 compared with the present year come in the Department of Energy (down 7 per cent), the Department of Environment (down 9 per cent) and significantly (as it spends half the government budget in this area) the Department of Trade and Industry (down 8 per cent).

Defence research and development, however, will be maintained at constant prices to 1986-87, although even these large sums (at £7,077 million in 1984-85 some 50.3 per cent of all government-funded research and development) are falling as a fraction of defence procurement.

The Cabinet Office also takes a special sectoral look in its report at British spending on marine science, in an attempt to identify "gaps and overlaps" in spending; but here it prepares the ground, unfortunately, for more cuts. "There seems cause for considering" write the authors "whether the present provision of facilities [15 towing tanks, 7 cavitation tunnels, etc.] is excessive for a shipbuilding industry which declined 30 per cent between 1975 and 1981". Decline, it seems, begets decline.

Robert Walgate

UK science

Now you see it, now you don't

A MUCH-needed present for British science (generally regarded as being on its uppers) was divided and praised last Tuesday morning — but taken away, at least partially, in the afternoon. The moral of this strange story is that Britain's mechanism for allocating budgets between ministries is in need of drastic overhaul.

At noon on Tuesday, Sir David Phillips, the chairman of the Advisory Board for the Research Councils (ABRC), which each year allocates monies to its member councils, was praising the "courageous" action of Secretary of State for Education and Science Sir Keith Joseph in raising new money for science in 1985–86 and subsequent years. (He had promised an effective £16 million annually for the research councils by a reallocation of resources within his own cash-limited ministry, making well-off British parents pay a little more for their children's university education and giving this as a bonus to science.)

But by 3 p.m. the same day, Sir Keith was having to bow before a storm of protest in the House of Commons from what sometimes seems to be the only effective British political opposition, given the large Tory majority — the Tory back-benches.

Back-benchers had reacted with horror at what was really a fairly moderate increase in parental contributions to student grants among better-off families (see below). Sir Keith hung on to his principles (there will be a commission on student funding), reduced the new burden on parents, and was partly bailed out by the Treasury. The result was some £3 million less than expected for ABRC next year (and £6 million less for the "equipment grant" of the University Grants Committee). It was a "very disappointing" result according to a spokesman for one of ABRC's member councils, the Medical Research Council (MRC).

Science will, however, still get more than was promised before Sir Keith's grants saga began. The way the Department of Education and Science now explains the figures is this. Sir Keith's plan had been to eliminate the minimum student grant of £205 a year, to increase the parental maintenance contributions for family incomes above £11,500 and to impose tuition fees for incomes above £20,000. This would have released £60 million in a full year but only £39 million next (not a full year as the financial and academic years do not

match). Sir Keith has now been forced to cancel the imposition of tuition fees. This loses his department £21 million next financial year. The Treasury has offered £10 million, leaving £11 million to find within the department.

The effect on the research councils is a £3 million loss compared with expectations at noon on Tuesday. In the financial year 1985–86, ABRC was to have received £571 million (an increase on last year based on price indexation) plus a bonus from Sir Keith's grants plan of effectively £14 million (plus £2 million from a salaries adjustment). This bonus will be reduced to £11 million (plus the £2 million). An intended £10 million bonus on the UGC equipment grant is similarly lopped by £6 million, and £2 million is lost elsewhere.

The cut (and the bonus) may seem small, but on the original plans ABRC was to spend £2.75 million of the bonus on "restructuring" (effectively, pensions and redundancy). So the bonus amount for science is really falling from £13.25 million to £10.25 million, a large enough fraction that the horse trading over how to divide up the money among the research councils must begin again.

Discussions on this are taking place this week, when, unfortunately for the Science and Engineering Research Council (SERC), its chairman, Professor John Kingman, out of the country. Last week he had been describing his pleasure at the "enormously valuable" £8 million SERC was to have received. "Without this money we would have had to cut the number of the best (alpha) grant applications we could support to just 60 per cent of the applications", he said at noon last Tuesday, "but now we can raise it to 70 per cent". MRC equally was hoping to raise the same figure from the "disaster" of 53 per cent this year to "somewhere in the 60s".

Sir David Phillips has meanwhile been roundly condemning the almost entirely separate way in which the different departmental budgets are arrived at — so that the science budget of Department of Education and Science, for example, cannot benefit from savings, say, in the defence development budget (which accounts for a quarter of British research and development). The Department of Trade and Industry's research budget is also not "tradeable" with the ABRC sums. An infamous (if possibly apocryphal) case being quoted last week was the "heavy armoured vehicle" whose development in the Ministry of Defence cost hundreds of millions of pounds but whose doors would open only when the vehicle was level. The errors in the defence research and development budget alone, it was suggested, could bail out British basic and strategic research. But such balancing — which should take place in the Treasury as Britain has no overall science minister — never takes place. It is time for a change, it is being said more and more loudly in the scientific corridors of power.

Robert Walgate

Students granted a reprieve

SIR Keith Joseph's retraction of his proposal to make better-off parents contribute to their children's tuition fees means that individual parents will be up to £520 a year better off at an expense to the government of £21 million in England and Wales. To find the £11 million that the Treasury will not provide, Sir Keith has reduced by £3 million the proposed increase to the science budget, by £6 million the proposed £10 million increase in the universities' equipment grant and by £2 million the increased grants in the Professional and Industrial Commercial Updating Programme (PICKUP), adult education and the microelectronics programme.

An inquiry into the whole subject of students grants is to be set up by Sir Keith to avoid the same kind of embarrassing miscalculations as occurred last Wednesday. No date for the review has been set, nor is there any guarantee that it will be an independent inquiry, but it will include the controversial suggestion that loans be made to students for their tuition and living expenses. The National Union of Students (NUS) strongly opposes this idea, believing that it discriminates against students from poorer backgrounds and those studying for a degree that does not relate directly to a business or profession. The organization does, however, welcome the suggestion of a review; at present 46 per cent of students do not receive the full extent of the deemed parental contribution, and now that the

minimum award of £205 a year has been abolished, many students will receive nothing unless their parents support their decision to gain a degree.

The Committee of Vice Chancellors and Principals (CVCP) hopes to be included in any review of the grant process. Tired of the government ignoring detailed annual reports on how much students need to live on, CVCP intends to set up its own inquiry to advise the government in any case. As the last review on the subject was in 1962, the need for a new inquiry is pressing. NUS would like to see the whole concept of parental contributions abolished; under the present system, a student is not considered independent until the age of 25.

Just how the cuts in the science budget increases will be implemented is not yet clear. The Department of Education and Science is leaving the details to the boards concerned. This must be of small comfort to the University Grants Committee which meets tomorrow; originally it was to discuss how to distribute the extra £10 million awarded to it for equipment by Sir Keith, but now its task is to divide up the much smaller cake of £4 million extra. Instead of being able to grant equipment money to three "broad areas" of science, it will now have to decide on one such area. There is a general condemnation of "robbing Peter to pay Paul", where either the students or the research programme in Britain will be the loser.

Maxine Clarke

AIDS screening

False test results raise doubts

Washington

THE crash effort by the Public Health Service, announced in April, to develop a blood test for AIDS (acquired immune deficiency syndrome) has run into difficulties. A pilot trial carried out by the Food and Drug Administration (FDA) within the past month indicates that the test kits being developed by five different contractors show wide variations in their sensitivity and selectivity, and may be subject to significant false positive and false negative rates.

The test kits all use the ELISA (enzyme-linked immunosorbent assay) method for detecting the presence in the blood of the antibody to the AIDS virus, HTLV-III. In the recent pilot trial, the five companies were each supplied with approximately 3,000 samples from plasma donations and 3,000 from whole blood donations. According to researchers who have seen results from the plasma samples, the five companies obtained positive readings on a widely varying number of samples, from a low of 5 to a high of about 100 out of 3,000. If the 3,000 samples were representative of the general population, some 20 to 30 positives would have been expected.

Any interpretation of these findings, however, is complicated by the fact that the five companies each received a different set of 6,000 samples. Each company then ran its own confirmatory tests on the positives it obtained on the ELISA tests. These confirmatory tests, using the so-called Western blot technique (an independent and more selective test), suggest a false positive rate of one out of every ten that were scored positive by the ELISA test.

According to Lowell Harmison of the Office of the Assistant Secretary for Health, duplicate samples were not initially sent to each of the companies because one object of the trial was to assess the prevalence of AIDS in the population, a goal that required a larger number of independent samples. And he said that controls on the results will be obtained by having all positive samples retested by all five companies, as well as by an FDA laboratory. He added that it is misleading to derive a false positive rate from the comparison of the ELISA tests with the Western blot tests because a consensus has only this week emerged on the definition of a positive on the Western blot.

John Petricciani, head of FDA's division of blood and blood products, which was in charge of the pilot trial, acknowledged that the trial shows that "some companies have more work to do than others in refining their tests", but that the primary purpose of the trial was to provide "an early look" at the performance of the test kits. Despite the setbacks, the Public Health Service is still predicting that the first tests will receive FDA approval and be on the market in the first quarter of 1985.

Some 14 million units of blood are collected each year in the United States from about 3-4 million individual donors. A false positive rate of 1 in 10 positives, combined with an overall incidence of 1 per cent, implies that as many as 4,000 donors would be incorrectly identified as having AIDS antibody each year.

Dr Peter Page, director of the American Red Cross blood services for the north-eastern United States, said he was concerned that the tests may be rushed to market before this false-positive problem is corrected. He said that any test goes through a development stage, in which problems are identified and resolved, but that "we're being rushed so much by Margaret Heckler [Secretary of Health and Human Services] that we don't have time

to resolve them" in the case of the AIDS test. Page noted that appeals to members of high-risk groups voluntarily to abstain from giving blood have already significantly improved the safety of the blood supply; there is no compelling case to rush ahead with blood tests that would "add a relatively small increment of safety", he said.

Although some improvement may come with better calibration of the ELISA tests, the test may prove to be essentially problematic. The test uses virus antigen to detect antibody; preparation of pure antigen is complicated by the presence of the cells used to grow the virus and the tendency of the fragile antigen-bearing coat of the virus to be lost in processing. In addition, some carriers of AIDS virus may show no antibody response whatsoever. A more advanced approach to detection might be a test that looks for the virus antigen directly in the blood.

Stephen Budiansky

British AIDS

Paper prophylaxis backfires

GOVERNMENT assurances about steps to combat the spread of AIDS (acquired immune deficiency syndrome) in Britain seem to have done as much to worry as to reassure people. One part of the British government's plan is the new Blood Products Laboratory at Elstree, north of London, opened two weeks ago by Mr John Patten, Minister of Health, which can, among other things, produce factor VIII, the blood-clotting factor used in the treatment of haemophilia.

But David Watters of the Haemophilia Society is not convinced that Elstree can meet the demand. "What the United Kingdom needs to do is invest in plasma research", he said. The total amount of plasma donated in England and Wales accounts for only 30 per cent of the annual requirement. On the same day that Mr Patten spoke of Elstree being self-sufficient, he announced further plans for heat-treated factor VIII. A spokesman for the Department of Health said "at least the means to become self-sufficient will be there". But, as David Watters points out, "it's no good having all these factories and nothing to process through them".

Meanwhile, according to Mr Patten, the war against AIDS is being waged on four fronts. The Medical Research Council (MRC) is supporting research projects at St Mary's Hospital, London, on the immunology of AIDS, at the Institute for Cancer Research on retroviruses associated with AIDS, and at the Middlesex Hospital. The MRC working party on AIDS, under the chairmanship of Dr David Tyrrell of the Common Cold Unit, was set up in the summer of 1983 and co-ordinates these projects. One of its members, Dr Richard Tedder of the department of virology at Middlesex Hospital, told a conference organized by the Terence Higgins Trust a

week ago that monogamy is the only way to restrict increasing incidence of the disease. There is some evidence that the homosexual community is taking this advice to heart, according to a survey published in the *British Medical Journal* in October.

The Communicable Disease Surveillance Centre at Colindale in London constitutes the government's second front, collating all the available information on the disease and its incidence.

In the area of public education, events move sluggishly. The Department of Health is now reprinting a leaflet circulated by the National Blood Transfusion Service (NBTS) because an earlier version discouraged from giving blood only homosexual men prepared to describe themselves as promiscuous; the new version discourages homosexual and bisexual men altogether. People who have taken drugs, visited Haiti or had any connections with central Africa (where AIDS may have originated) are also excluded.

Something similar has happened to the Health Education Council's pamphlet, *Some facts about AIDS*, due out in January 1985. Leaflets are, of course, only as effective as their circulation. A receptionist at a NBTS centre was recently asked why no such warning to prospective blood donors was on display. "We did have them out", she said, "but they frightened our customers away". Dr Harold Gunson of NBTS admits to some haphazard distribution in the past, but promises "efforts will be made to ensure that all donors attending the clinic receive a copy".

Many of the problems faced by blood banks would be solved if a reliable screening test were available. The Department of Health claims that such a test has been developed by Dr Tedder's team at the Middlesex Hospital.

Hugh Barnes

Polish universities

Setback for liberalization

Warsaw

WARSAW University's new rector, Dr Grzegorz Bialkowski, a physicist and non-Party "moderate", was elected last week in place of the "liberal" Dr Klemens Szaniawski, whose election, last May, was vetoed by the Minister of Science, Higher Education and Technology, Dr Benon Miskiewicz. The elections took place under democratic procedures laid down during the Solidarity era, procedures which, it is feared, will shortly be modified to increase ministerial control.

The liberalization of higher education under Solidarity was embodied in a new Higher Education bill which was not in the event made law until May 1982, five months after the declaration of martial law. By that time a number of amendments had been introduced, strengthening the power of the minister to overrule university decisions. Last May, Dr Miskiewicz used these powers to veto the elections of the rectors of four institutions (Warsaw, Wroclaw and Poznan Universities and the Higher School of Fine Arts). When Warsaw University tried to resist, the minister simply suspended the university's electoral college for six months and extended the term of the retiring rector, Dr Kazimierz Dobrowolski.

Hopes that such ministerial interventions would be kept to a minimum were raised at the beginning of the current academic year, when it was announced that the suspension of the electoral council would be lifted and new elections held as soon as the six months had expired. This optimism, however, was considerably reduced a few weeks later when documents began to circulate in Warsaw and other university towns setting out what purported to be proposed changes to the 1982 Higher Education on Act, which would considerably curtail university autonomy. The alleged changes included: reduction of the election of rectors to a choice between two candidates nominated by the minister; strengthening of the powers of the minister to suspend lecturers and/or collegiate bodies if he deems that they have been acting "contrary to the interests of society"; virtual abolition of participation by students and junior staff in decision-making within the university; reorganization of the *samorzad*, the non-aligned students' "self-government body", on the basis of existing student and youth organizations, all of which are Party-orientated.

Such allegations, Miskiewicz announced, were entirely false and "provocative". Nevertheless, in a meeting with the Main Council for Science and Higher Education on 15 November, he outlined his own proposals for amending the 1982 act, which according to participants were simply the proposals of the "provocative" document

in somewhat less specific form. The Main Council, which consists of one delegate from every state institution of higher education in Poland, strongly opposed the changes, noting that the 1982 act is, for "a significant fraction of the university milieu", the symbol of academic autonomy, and urging that to change a law barely two years after it came into force can only lead to a sense of doubt and instability. Even the least disapproving of the Main Council members are said to take the view that the law should be allowed to stand at least for another three years (a rector's term of office) and that any amendments should then be based on consultations and open discussions.

Students, of course, are particularly perturbed by the proposed reform of the *samorzady*, which would effectively

transfer into Party hands both such consultations on university policy as would still be left to the students after the reforms and also a whole range of welfare and social matters. Although the 1982 act forbids the formation of *samorzad* structures above the level of the individual university, representatives of the *samorzady* of all Polish universities and high schools met recently in Poznan to discuss the threatened changes. This technically illegal meeting was allowed to take place, although all participants had their identity documents checked by security police patrols in the vicinity of the assembly point. Although the content of the meeting remains confidential, it is understood that the *samorzady* as, for the moment, legally-constituted organizations, hope to be able to voice their concern through the peaceful consultative procedures provided by the 1982 act, and to restrain the more radical student elements from such proscribed activities as strikes and demonstrations. **Vera Rich**

European cooperation

All-optical computer in sight

Brussels

THE European all-optical computer is just around the corner or, more precisely, less than a year away. Following a major development in optical logic gates, demonstrated in London in November and in Brussels last week, the prototype of the all-optical computer is to be built at Edinburgh's Heriot-Watt University by a team headed by Professor Desmond Smith.

The operation is part of two-year project in the field of all-optical information processing based on the concept of optical bistability. Eight research teams have collaborated in the European Joint Optical Bistability Project, which will cost 1.8 million European Currency Units (ECU) — about £1 million. They work at the Université Libre de Bruxelles (Belgium); University College Dublin (Ireland); the Centre National de la Recherche Scientifique in Strasbourg (France); the Universities of Milan, Pisa and Florence (Italy); the University of Frankfurt, the Fraunhofer Institute of Instrumental Physics in Freiburg, the Max Planck Institute of Quantum Optics in Munich and the Technical University of Berlin (West Germany); and the Royal Signals and Radar Establishment, Malvern (United Kingdom). The teams were brought together by the Community's Committee for the European Development of Science and Technology (CODEST) which promotes cooperation between research institutes and scientists.

In *Nature* last January (307, 315; 1984), Professor Smith described new developments in optical bistability and the coupling of optical logical gates, in the infrared spectrum at cryogenic temperatures, which can switch with milliwatt laser beam power

and give sufficient output to switch further interconnected elements.

The latest breakthrough involves the coupling of optical gates at visible wavelengths using the semiconductor zinc selenide, one of the many new materials tested, at room temperature. The immediate advantage of the process is that it greatly facilitates construction and could revolutionize computer design. It could also be used in arrays and displays.

The technology is similar to that used in interference filter technology: the thermally-evaporated thin films are cheap and easy to produce and, being uniformly thick, can be used, unlike to earlier technology, for large array systems and two-dimensional devices.

The disadvantage is that the use of thermal effects slows down the process but, says Professor Smith, the possibility of having many elements in parallel will increase overall speed. Limitations, he says, are now only likely to be in the order of sub-microseconds or even tens of nanoseconds.

There will be benefits in processing information from radar arrays, sonar devices and video signals, he says. Eventually it might be possible to make serial parallel conversions and to store signals in optics for longer periods of time, in fact to improve all computing processes that benefit from parallelism (for example, pattern recognition input devices reading information in parallel). Optically bistable devices could also be a better means of translating fibre optics signals.

According to Professor Smith, it will now be possible "to begin to construct digital optical circuits more complicated than demonstrated with two elements and go on from there to create optical computer architecture". **Anna Lubinska**

Fast reactors

Britain falling behind?

BRITAIN'S nuclear engineers were making a strong plea last week for a British commitment to the development of fast reactors — little over a year after the government announced there was no need for haste. Nothing has changed, of course, in the engineers' perception of need: they say Britain needs the reactor as part of an arsenal of energy sources for the next century. What has changed, however, is the engineers' perception of politics. Since January, Britain has been signatory to a five-nation European umbrella agreement on fast reactor research and development, and there are growing fears that Britain will become second fiddle to France.

The plea last week came from Dr Tom Marsham of the United Kingdom Atomic Energy Authority (UKAEA), at a dinner for members of the British Nuclear Energy Society and British Nuclear Forum, that "we need to follow present plans enthusiastically and avoid further delay".

Whispers at the tables, however, indicated that the future may be with TOR, a new fast-fuel reprocessing plant under construction at Marcoule in France. Until TOR, Britain was generally thought to have the lead in efficient fast-fuel cycle management with a plant at Dounreay.

The preference for TOR gains significance in the context of an emerging consensus that Europe should develop just one major fast-fuel reprocessing plant to cope with all the fuel from the three commercial demonstration fast reactors (CDFRs) that are ultimately expected to be built in Europe (in France, West Germany and the United Kingdom). Since it is expected that Britain will have the last of the three CDFRs, it would be convenient, at least to British nuclear engineers if Britain were to win the reprocessing plant. If nothing else, it would help UKAEA to attract the engineers it needs.

Meanwhile, however, all is not going entirely smoothly on the European mainland. Electricité de France is not happy with the £2,000 million costs of Superphenix, the 1200 MW French CDFR due to start producing electricity at the end of next year, and sees no immediate prospect of further investment in that area.

And in West Germany, the increasing political power of the Greens, the environmental grouping which has now allied with the Social Democratic Party, may prevent any further fast reactor developments (such as the proposed CDFR, SNR 2).

Robert Walgate

Space station

Budget cuts spell delay

Washington

SPENDING cuts proposed by President Reagan in his effort to limit the federal budget deficit without increasing taxes are likely to delay completion of the space station now under development by the National Aeronautics and Space Administration (NASA). NASA services are among the targets listed in a budget plan presented to the cabinet last week, and NASA officials hold out little hope that the agency can escape unscathed at a time when, for example, one cost-reduction measure under consideration is a 5 per cent pay cut for all civilian employees of the federal government — which would, incidentally, also include NASA scientists.

Last year, NASA was told to expect 1 per cent real growth per year between 1986 and 1989 in order to allow development of the space station. Although NASA has traditionally been relatively immune from budget restrictions, the scale of the savings now being sought — \$34,000 million in fiscal year 1986 — means that few agencies can be permitted unnecessary increases. A decision to abandon the 1 per cent annual growth target would leave NASA administrator James Beggs with some hard decisions.

Although President Reagan is publicly committed to the space station project, the project is still in a very early stage of

development and could be postponed without incurring huge additional costs. A recent unfavourable report on the space station from Congress's Office of Technology Assessment might increase pressure for the project to be put back. One difficulty, however, is that when the President announced his plan for a permanent manned space station, in January 1984, he directed NASA to complete development "within a decade". A decision to abandon that target would therefore have to be approved by the President. Initial development costs for the space station — without instrumentation — are put at \$8,000 million.

Other contenders for programme delays are scientific projects for which funds have not yet been approved. Possibilities include TOPEX (Topographic Experiment), an ocean surveying satellite, and the advanced X-ray astrophysics facility planned for the 1990s. The Mars geoscience/climatology orbiter has also been suggested.

The Hubble space telescope, now nearing completion in time for a 1986 launch, is unlikely to be affected by any budget reductions, say NASA officials. Although the project has been subject to many delays due to technical difficulties and budget stringencies, there are now no outstanding obstacles.

Tim Beardsley

Nature Conservancy

Chief geologist resigns

THE British Nature Conservancy Council (NCC) was both criticized and rewarded last week. Dr George Black, NCC's chief geologist for the past 24 years, resigned because he considered recent developments in NCC policy alarming in their "uncompromising militancy". The government, however, has made it clear that it will support a private member's bill, tabled by David Clare, the opposition spokesman on the environment. The bill seeks to arm NCC against landowners who exploit of an anomaly in the Wildlife and Countryside Act of 1981 and destroy their land during the three months before its registration as a special, protected site. If the bill becomes law NCC will be further empowered to block any work on a prospective site that might be harmful.

In accordance with the terms of the Wildlife and Countryside Act, NCC is resurveying the 4,000 Sites of Special Scientific Interest (SSSIs) and "renotify" owners and users of their obligations under the act, a costly and labour-intensive process. Earlier this year it was claimed that NCC has neither the money nor the manpower to carry out these duties but an increase of 50 per cent in its grant-in-aid budget of £7 million has recently been announced.

Dr Black's resignation, however, has been induced by the council's declared intentions on the selection of additional sites, and its strategy for the future, as published in *Nature Conservation in Great Britain* last June. Dr Black regards conservation as an applied science. "Previously, NCC saw its role as the government's scientific adviser on the natural environment. The report is beginning to introduce other concepts, such as conservation as a cultural matter." According to Dr Black, this marks a departure from its long-established tradition. In a farewell letter to his staff, Dr Black explained that differences grew out of NCC's commitment to the conservation of "a flood of scientifically sub-standard SSSIs for cultural, recreational, inspirational and spiritual reasons". Despite his anxiety to correct press reports that have presented the disagreement rather melodramatically, in terms of feuds and bad blood, Dr Black is resolute. "I've come to the conclusion, as a scientist, that I want to work for a purely scientific organization."

As far as departures from tradition go, some council members feel that NCC's earlier practices were ineffective and in need of change. In the past few years, NCC has been moving not towards confrontation and *diktat*, but very approximately into line with the opinions of the general public who finance it.

Hugh Barnes

Soviet space programme

Halley mission on course

THE era of exploration of Halley's comet is at last beginning to unfold. In two days' time, the first of a clutch of satellites due to pass by Halley sometime in March 1986 should be launched. VEGA, or "Venus-Halley", a Soviet-French collaboration which will drop probes into the atmosphere of Venus on the way to Halley, should get away this Saturday.

One French participant described VEGA recently as "a fantastic mission, really gorgeous". Soviet space technology is sometimes rough-and-ready in comparison with that of the United States, but VEGA has some beautiful equipment, according to Jean-Loup Berthod of the Verrières-le-Buisson space laboratory of the Centre National de la Recherche Scientifique.

For instance, VEGA will carry the first planetary mission camera equipped with an advanced charge-coupled-device (CCD) imaging surface, says Berthod. CCDs are "much more flexible" and linear than the standard "videcon" detection system, and "the future for space exploration is CCD" says Berthod. The US Galileo trip to the outer planets will use CCD imaging. But VEGA will be first, using a camera built by the Soviet Union, Hungary and France and with a Soviet CCD. French laboratories have made calibrations of the device and "at the present time it seems to be good".

There will be two VEGAs with identical payloads, the second to be launched a few days after the first, and each should arrive at Venus in June next year. Each VEGA will then divide into two parts, one to fly on to Halley and the other to descend onto Venus ("the Soviet's pet planet", as Berthod describes it).

Each VEGA descent probe should eject a small balloon carrying instruments to measure local pressure and temperature, and a small radio transmitter which will be followed on Earth for some days by very long baseline radio interferometry (VLBI) with the US Deep Space Network and the French and Soviet networks. This will enable the first measurement of wind speeds and directions in the venusian upper atmosphere.

The French interest has been mostly to measure a sulphur dioxide profile through the atmosphere, to learn if it may be concerned with volcanic activity. Pioneer V indicated a tenfold decrease in upper atmosphere concentrations of the gas, suggesting that the high levels detected earlier may have been transitory.

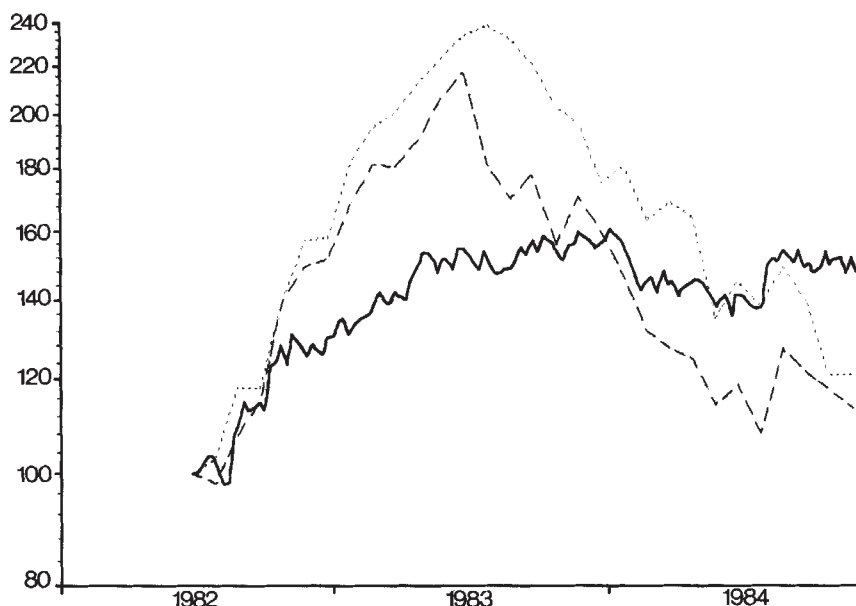
As for Halley, the first VEGA probe should reach the comet on 8 March 1986, five days before the European mission Giotto. VEGA-1 is due to pass at 10,000 km from the cometary nucleus, following the nucleus with the CCD camera on a tracking platform. VEGA information will be relayed to the European Space Agency

to help it to make the last targeting adjustment to Giotto, which is due to go much closer — within 500 km on the sunlit side and into the dust cloud. VEGA-2 will pass at 5,000 km, but it has not been decided yet whether it will go before or after Giotto (which is quite likely to be destroyed in the encounter).

"The Soviets wanted survival, and so had to go outside the large dust cloud", says Berthod. And they also wanted good pictures of the nucleus. Hence the CCD camera, and hence also the distance of approach. At a fly-by velocity of some 70 km per second, the rate at which the camera could be turned to keep track of the nucleus fixed a fly-by distance of a few thousand kilometres. Giotto will also attempt to track the nucleus, but by turning a lighter mirror system.

Robert Walgate

Comparison of biotechnology stock indexes



— New York-Dow Jones Industrials Price Index
 Nature Biotechnology Index
 --- Hambrecht & Quist High Technology Index

A comparison of the *Nature* index of biotechnology stocks (which started at 100 on 25 June 1982) with the Dow Jones and Hambrecht & Quist indexes to November 1984. (Source: Datastream/Peter Laing of Biotechnology Investments Limited.) □

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 30 November	Change
14	5 3/4	Biogen (Switzerland)	7	5 3/4	-1 1/4
2	1	Bio-Logicals (Canada)	1 5/16	1 3/8	+1 1/16
14 3/8	4 1/2	Bio-Response (USA)	6	4 1/2	-1 1/2
14 3/8	8 3/4	Cetus (USA)	10 1/4	8 7/8	-1 3/8
10 3/8	4 1/4	Collaborative Research (USA)	4 1/2	5 1/4	+ 3/4
19 7/8	11 1/2	Damon (USA)	12 1/8	11 3/4	- 3/8
26 1/4	11 3/4	Enzo-Biochem (USA)	18 1/4	15 1/8	-3 1/8
10 1/8	3 5/8	Flow General (USA)	4 7/8	3 5/8	-1 1/4
42 1/4	28 3/4	Genentech (USA)	28 7/8	33 1/4	+4 3/8
10 3/4	4 1/2	Genetic Systems (USA)	6 1/2	6	- 1/2
17 1/4	6 5/8	Genex (USA)	7 1/8	6 5/8	- 1/2
23	11	Hybritech (USA)	15 3/4	15 1/4	- 1/2
16 1/4	6 1/4	Molecular Genetics (USA)	7	6 1/4	- 3/4
15 1/2	8 1/4	Monoclonal Antibodies (USA)	10 1/2	9	-1 1/2
60 7/8	20 7/8	Novo Industri A/S (Denmark)	20 7/8	24 1/2	+3 5/8
22 3/4	14 1/2	Pharmacia (Sweden)	16 1/4	16 1/8	- 1/8

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature's* weighted index of biotechnology stocks stood at 121 on 30 November, the same as a month earlier. Data from E.F. Hutton, Inc.

Teller's cold comfort

SIR — Turco *et al.*¹ have performed a valuable service by bringing to public and political attention the probability that, in addition to the "known" effects of blast and radiation, large-scale nuclear war will cause severe climatic disturbance which could increase the number of fatalities. Expressed in those simple and general terms, their claim is unassailable but, without supporting argument, it would attract neither scientific nor political interest nor would it be intellectually satisfying to the authors.

Because there was detailed supporting argument, criticism of their assumptions and model was inevitable. Predictably there has been a rapid succession of publications describing alternative analyses of the likely climatic consequences of global nuclear war, no doubt motivated in part by an understandable academic desire to arrive at the correct conclusion and by some of the other attractions of publishing in the vanguard of a topic that is attracting wide public interest. I should emphasize that this motivation is academic rather than scientific since the proponents of the various models have less chance of testing them experimentally than proponents of some of the more esoteric theories of cosmology.

Of the articles I have seen, the one that alarms me most is that of Teller². It is well argued, as one would expect, and apparently well supported factually. It is soothing in a way that the arguments from supporters of the nuclear industry often are. ("When the biological recovery formula is applied, a dose rate as high as 250 rem from worldwide fallout is built up at a rate so slow that it would not produce significant casualties". I do not wish to seem ungrateful, but this information does not make nuclear war any more appealing.)

In his introduction, Teller says: "Scientific knowledge of the after-effects of a nuclear war ... is of great importance in making political decisions". In his conclusion: "Therefore there is every reason to undertake the difficult task of arriving at more realistic predictions. ... Greater amounts of money, carefully spent on atmospheric modelling and experiments, would accelerate resolution of the basic questions regarding nuclear winter."

This, of course, is the substance of a cost-benefit analysis of nuclear war. With appropriate correction for distribution of bombs, the relation between mass of bombs exploded and the number of resulting deaths presumably resembles the Beer-Lambert spectrophotometric relation with a linear component for few bombs and a flattening of the curve for many bombs. If the "political decisions" of which Teller writes (I assume he means whether or not to bomb the Soviet Union) were to make any military sense, then the number of bombs used would have to be

small enough to ensure that they were on the linear part of the death curve. Notwithstanding Teller's point about accurate low-yield missiles, that possibility seems very remote. Moreover, the correspondence about nuclear winter is specifically based on many bombs.

If it could be demonstrated that the predictions of a nuclear winter are exaggerated, how would that knowledge be used? Would one side bomb the other forthwith, secure in the knowledge that their few survivors would not be unduly cold, at least in summer? Or conversely, if the prediction is not exaggerated, does that mean that each side should stockpile food, as Teller suggests, so that the survivors can at least have some cold, albeit radioactive, porridge? If, in more comprehensive terms, the additional research for which Teller calls should conclude that a global war would not kill everyone, but merely 90 per cent of the world's population, would that make the war acceptable? The fine details of the ostensibly rational discussion about nuclear winter are fast making that discussion the penultimate insanity.

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Bedlam at banquets

SIR — At many international congresses — phycological, entomological, and all sorts of others I've attended in recent years — the value and pleasure of the closing dinners have been considerably reduced by noise. Bands and comedians, however good, and however appropriate for youth or political rallies, are out of place at scientific meetings, particularly on the final evening which may be the last (or even the only) occasion for congenial and informal discussions.

Musicians and other entertainers, with their inevitable kilobel amplifiers, should not be included in the programmes on such occasions. Communication is hard enough as it is, especially for the partly deaf, or for Finns and Turks trying to discuss technical matters in English with Japanese and Chinese colleagues who have gathered, often at considerable expense, perhaps for the only occasion in their lives; gratuitous noise makes it harder. There is a place and a time for entertainers, but not at conferences which we attend principally for the business of conferring.

Organizers, please note.

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Plight of UK scientists

SIR — The plight of Indian scientists (*Nature* 8 November, p.87) should not blind us to the problems which British scientists have long faced. In the words of your leading article, India has had a government whose policy for science had open and straightforward objectives, namely that understanding the natural world would in due course be a source of economic prosperity, social improvement and the banishment of superstition. Britain, by contrast, has a government which has looked mainly to immediate industrial benefits from science, and has adopted a policy towards higher education that is resulting, whether the government realizes it or not, in the Dissolution of the Universities. Research has not only been stultified by lack of funding in other than a few favoured subjects, especially in our universities which should be the power-houses of research, but has also been subjected to policy planning which, by its very nature, cannot but direct resources and effort to the last problem instead of the next one. Desperate though the need for improved financial support is, it is even more vital to return to the tested and time-honoured Haldane principle in research.

Perhaps the international scientific community can indeed help science in India, but reciprocally science in Britain would be enormously helped if our national decision-makers were to learn from India.

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Peer review

SIR — Surely, if a "considerable element of chance is an inescapable component of today's review procedures" (Bryn Bridges, *Nature* 4 October, p.406), then the system should have been designed to take account of this random element. For example, the sharp cut-off point in the rank order when funds are exhausted would be blunted if there were a sliding scale of funding. Progressively less outstanding applications would receive progressively less than the optimum funds required to complete the project in a reasonable time. Applicants would then have to modify or slow down the rate of work. At least the entire project would not collapse. The peer review system was designed in the "good old days", when virtually any good application was sure of being funded. The system has failed to adapt to the realities of funding today's research^{1,2}.

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Opportunities in particle physics

from C.H. Llewellyn Smith

While a British committee is considering the question whether the United Kingdom can afford to remain a member of the European high-energy physics consortium CERN, particle physics itself is at an exciting stage. Here a British theorist describes the prospects for the coming decade.

THE spectacular advances in particle physics of the past fifteen years culminated in the discovery of the W and Z bosons, the carriers of the weak force, in experiments at CERN last year. These experiments have now produced tantalizing hints of quite unexpected new phenomena. This is therefore an exciting time for particle physics, and a good one to assess the progress that has been made and to look to the future.

How far ahead is it sensible to look? It is sometimes assumed that the cost of accelerators is growing inexorably so that, sooner or later, particle physics must grind to a halt. The facts tell a different story. In the case of CERN, for example, the cost of the LEP machine (the large electron-positron collider), which will come into operation in 1989, is about three-quarters of the cost of the previous accelerator, the SPS, commissioned in 1977. In fact, the total CERN budget, which includes the operation of existing facilities as well as capital costs, has fallen by about 20 per cent in real terms during the past decade, from a peak in 1974 during the construction of the SPS, and will remain constant during the construction of LEP. Looking beyond LEP to the end of the century, it seems that a very powerful proton collider could be built in the LEP tunnel without increasing the CERN budget, if that should prove desirable.

The past two decades

In order to understand the present state of particle physics, it is instructive to begin by recalling the situation twenty years ago. The known constituents of matter could be divided into two classes:

- **Leptons.** These are particles such as the electron, the muon and neutrinos which do not feel the strong, or nuclear, force. Four were then known, and it was clear that they were simple objects with no size, within the limits of experimental resolution.

- **Hadrons.** These are the particles such as the proton, the neutron, the pi-meson or pion, hyperons, and so on, which do feel the strong force. Hundreds were known and it was clear that they were complex objects with finite sizes.

It was known that four forces control the behaviour of these particles:

- **The gravitational force.** Gravity has been well understood classically since Newton's theory was perfected by Einstein, but even now there is no satisfactory quantum theory of gravity. The effects of gravity are quite negligible in experimental particle physics, and are important only for very large bodies, because gravity acts in-

discriminately and additively on all matter. Theoretically, however, it may be that we ignore gravity at our peril when studying the nature of force.

- **The electromagnetic force.** Electromagnetism is described by Maxwell's equations and Dirac's theory of the electron, which were successfully combined to form quantum electrodynamics, or QED, in the late 1940's. The electromagnetic force holds atoms together and controls the behaviour of matter in bulk.

- **The strong force.** This is responsible for holding the nucleus together; its origin was obscure twenty years ago.

- **The weak force.** This is responsible for β -decay and other rare processes. No mathematically consistent theory of this force was known.

As can be surmised from this brief summary, the immediate goals of particle physics were to determine (1) the nature of hadrons and the relationship between them, (2) the nature of the strong force, and (3) the nature of the weak force. Two decades later, it can be argued that all these goals have been achieved.

As the spectrum of hadrons was slowly established by many meticulous experiments, patterns began to emerge and it was shown that they could be explained by assuming that the hundreds of hadrons are composed of a small number of more fundamental entities, called quarks, put together in the different ways allowed by quantum mechanics. Experiments using high-energy electrons (at SLAC in California) and neutrinos (at CERN) made it possible, in effect, to X-ray the proton, revealing that it does indeed contain objects with exactly the properties previously postulated for quarks. The unexpectedly large probability for backward scattering found in these experiments revealed that quarks, like leptons, are point-like within the resolution, just as the observation of large back-scattering of α -particles by gold revealed to Rutherford that the nucleus is an extremely small part of the atom.

Once it was known that hadrons are composed of quarks, it became possible to construct a theory of the strong force. This force acts between quarks, which it holds together to form hadrons, generating nuclear binding as a secondary effect. The data led to a theory known as quantum chromodynamics or QCD.

According to relativistic quantum mechanics, all forces are generated by the "exchange" of particles. An analogy is provided by two skaters exchanging a ball while gliding on a frozen lake, who would

be forced apart by the impulses generated when they catch and throw the ball. (This analogy fails to explain how forces of attraction are generated, although the exchange of boomerangs might be invoked!) In QED, for example, the electromagnetic force is generated by the exchange of photons between electrically charged particles. Weak processes are generated by the exchange of the heavy W and Z particles. The interquark force is generated by the exchange of particles called "gluons". In QCD a quantity known as "colour" plays a role analogous to that of electric charge in electromagnetism (hence the name chromodynamics). Both quarks and gluons carry colour charge, so gluon-exchange generates a gluon-gluon force as well as gluon-quark and quark-quark forces. It seems that these forces are so strong that quarks and gluons cannot exist as individual particles, but always bind together in combinations with zero colour charge to form hadrons.

It is much harder to wrest quantitative results from QCD than from QED, because of the strength and nonlinear nature of the interactions, but various qualitative results can be derived and some quantitative predictions have been made which have been borne out by experiment. One example is the prediction that, in certain circumstances, quarks should radiate energetic gluons which would give rise to characteristic collimated jets of particles. Such jets were discovered in experiments at the electron-positron collider PETRA in Hamburg, which revealed the existence and nature of the gluon.

Not long before the development of QCD, 't Hooft demonstrated the consistency of a theory of the weak force previously proposed by Glashow, Weinberg and Salam. Consistency requires this theory to be based on a principle known as local gauge invariance, which also underlies QED, QCD and Einstein's theory of gravity. In the case of gravity, local gauge invariance means that the form of the laws does not depend on the choice of the space-time coordinate system chosen to label events, and it actually allows different coordinate systems to be used at different places. In the other cases, local gauge invariance allows certain "internal" coordinate systems to be chosen differently at different places. In QCD, for example, each quark has one of three possible colour charges: the forces depend on the relative values of these charges, but not on which colour charge is assigned to a particular quark, and the assignment of "colour

coordinates" can be different at different places. The discovery that all the known forces are based on the same principle, which implies that they have a similar mathematical structure, opens up the exciting prospect of finding a theory that unifies all forces, in the sense that Maxwell's equations unify electric and magnetic forces.

The new theory of the weak force necessarily involves a partial unification with electromagnetism. In addition to the electrically charged W , whose exchange generates all the weak processes known before 1973, it requires the existence of an electrically neutral "heavy photon", called Z , and predicted a new class of weak processes that would be generated by Z -exchange. The discovery at CERN in 1973 of some of these processes, known as "neutral current neutrino interactions", was therefore a vital first step in establishing the electroweak theory.

The effects of a force are proportional to the strengths of the charges involved and also depend on its range. The analogy of the skaters passing a ball back and forth suggests that the heavier the carrier, the shorter the range over which it can be exchanged. In fact, the range of a force varies inversely with the total energy carried by the exchanged particle, including the energy (mc^2) stored in the mass. Because the mass of the photon is very small, certainly less than 10^{-30} kg and very probably zero, it can carry a negligibly small amount of energy. Consequently, electromagnetic forces can act over very large distances, as anyone who has used a compass knows. The basic idea of the unified electroweak theory is that the intrinsic strengths of weak and electromagnetic charges are similar, weak effects being relatively very feeble because the W and Z are very heavy and therefore act only over very short distances. If weak charges are indeed related to electromagnetic charges as in the unified theory, the observed weak effects require the W and Z to be about 100 times as heavy as the proton. Until last year, the data were consistent with any masses from 20 times that of the proton to infinity. The discovery of the W and Z with the predicted masses was therefore a major step forward as it provided the first direct evidence for electroweak unification.

The outstanding questions

The description of the structure of matter in terms of a relatively small number of quarks and leptons, whose interactions are controlled by QCD, the unified electroweak theory and by gravity, has come to be known as the "standard model". Although this model successfully describes an enormous body of data, it was recognized from the outset that it is incomplete. It simply does not explain enough. Too many of its ingredients are added in amounts adjusted to fit the data (although, in fairness to the model, it

The six leptons

Three electrically charged leptons are known: the electron (e), the μ (discovered in cosmic rays in the 1940s) and the τ (discovered at SLAC in California in 1975). Each is linked by the weak force to its own much lighter (possibly massless) neutral lepton or neutrino (ν) — so W exchange transmutes e into ν_e : $e \leftrightarrow (W)\nu_e$ and so on. As far as is known, the properties of the three "lepton doublets"

(e, ν_e) (μ, ν_μ) (τ, ν_τ)
are identical except that their masses differ. Their masses in GeV, in which unit the mass of the proton is 0.938, are

e : 5.1×10^{-4}	ν_e : less than 5×10^{-8}
μ : 0.106	ν_μ : less than 5×10^{-4}
τ : 1.78	ν_τ : less than 0.16

The six quarks

Six quarks are known. The up (u) and down (d) quarks are needed to make neutrons and protons: they move inside hadrons with effective masses of about 0.33 GeV. The strange (s) quark, with mass about 0.5 GeV, is needed to make the "strange" particles discovered in cosmic rays in the 1940s. The charmed quark (c), with mass 1.5 GeV, is needed to make the J/ψ particle, discovered at BNL on Long Island and at SLAC in 1974, and charmed particles found at SLAC in 1976. The bottom (b) quark, with mass of 4.8 GeV, is needed to make the T found at Fermilab near Chicago in 1977 and "bottom" particles found at Cornell in 1983. Finally, the top (t) quark is needed to make the standard model work, given the existence of the b and of six leptons. The 'UA1' experiment at the CERN proton-antiproton collider has recently discovered an exotic class of events which can be consistently interpreted as evidence for the t with a mass of about 40 GeV. All the quarks seem to respond identically to the strong force. The weak force links pairs of quarks, as it does leptons, thus

(d, u) (s, c) (b, t)

However, "off-diagonal" weak transmutations such as $s \leftrightarrow u$ and $b \leftrightarrow c$ occasionally occur: the standard model can accommodate but does not explain the existence of these transmutations. Likewise it can accommodate but not explain the observed numbers and masses of quarks and leptons. $\square$

should be acknowledged that most of these ingredients are quantities such as the fine structure constant and the ratio of the electron and proton masses which have hitherto been regarded as given fundamental constants). If the standard model is incomplete, its predictions have only a limited domain of validity, just as the predictions of Newton's theory of gravity, which is an approximation to Einstein's more complete theory, are not valid in all circumstances. Experimental particle physicists are therefore searching for phenomena that the standard model cannot explain, while theorists are striving to construct more complete models and predict what these phenomena might be.

Three major questions are raised, or not properly answered, by the standard model:

● **What determines the number of quarks and leptons?** At present six quarks and six leptons are known (see box). As far as we can tell, the present Universe would continue to function in much the same way

with just two of each, although six or more are needed in order to explain a phenomenon called CP violation which, although very rare today, is believed to have played an essential role just after the big bang in generating the excess of matter over antimatter now observed.

The numbers and spectra of quarks and leptons are essentially arbitrary in the standard model, and attempts to find more complete theories which explain them have, so far, been woefully unsuccessful. There are many other questions raised by quarks and leptons. Are their properties connected, or is it just coincidence that, for example, six of each of them are known, or that the electric charge of the electron is an integral multiple of the charges of quarks to better than one part in 10^{20} ? Will they turn out to be composite, which might account for their number and explain these parallels? History suggests the answer yes, but there is no hint of compositeness in their properties, whereas the composite nature of neutrons and protons was suggested rather directly by their sizes, anomalous magnetic moments and the existence of excited states.

● **Can we further unify the forces?** This is an obvious aspiration, given the success of the unified electro-weak theory and the discovery that all forces have a similar mathematical structure. A first step would be to unify completely the electromagnetic and weak forces, which are actually mixtures of two independent fundamental forces according to the standard model. The fact that the charges associated with the two forces turn out to have similar strengths certainly suggests that they are not in fact independent, but are linked in a more complete theory.

Complete electroweak unification is actually quite hard to achieve except in the much more ambitious framework of "grand unified theories" (GUTs), which not only fully unify the electromagnetic and weak forces but also combine them with the apparently very different strong force. GUTs depend on the fact that "charges" (strong and electroweak) are distance-dependent because of "vacuum polarization" due to the clouds of "virtual" particles which surround any charge. This is analogous to the screening effect of polarization in a dielectric medium, which causes the charge of a test particle to be effectively reduced (by the inverse of the dielectric constant) at distances greater than the molecular spacing. In QCD, there is anti-screening and the effective strong charge of a quark decreases as it is approached. It is therefore conceivable that at very short distances, inside the effects of all screening, the fundamental strong and electroweak charges are the same. Extrapolating their known values to very short distances, it seems that the strong charge and the charges associated with the two parts of the electroweak force do indeed converge. Conversely, the GUT hypothesis leads to a successful

post-diction for the relative strengths of the two parts of the electroweak force. From the distance at which the charges appear to converge, it can be inferred that the hypothetical particles, which are supposed to generate the screening masking the connection between strong and electroweak forces, must have masses about 10^{14} times the mass of the proton. These particles may produce dramatic effects which will be considered later.

In the absence of experimental evidence for their validity, GUTs are highly speculative, but at least they seem to be consistent. This is more than can be said so far of attempts to achieve the ultimate goal of synthesizing gravity with the other forces.

● **What is the origin of the masses of particles?** This question is brought into prominence by the success of the electroweak theory because the symmetries of the equations seem to imply that electromagnetic and weak forces are identical in range as well as strength, in which case the W and Z would be mass-less, like the photon. They also seem to imply that all quarks and leptons are mass-less, contrary to the facts. Nevertheless, the equations may describe the real world because they may have solutions in which the symmetries are hidden. Analogously, in a ferromagnet the atomic magnetic moments all line up in one particular direction chosen at random, thus hiding the rotational invariance of the laws of nature. In the standard electroweak theory, an added ingredient called the Higgs field causes the equilibrium state of the theory to be one in which the unobserved symmetries are hidden, thereby allowing the W and Z, and the quarks and leptons, to acquire masses. This theory can be made to fit the facts, but it appears to be very contrived. Is it only an approximation to a more complete theory, or may some quite different mechanism hide symmetries and thereby generate masses?

All the known ways of generating masses for the W and Z require the existence of new particles or new phenomena that may appear at energies which will be explored in forthcoming experiments — typically around 100 GeV (close to the rest energy, mc^2 , of the W and Z themselves) and in all cases below 1,000 GeV. (It is convenient henceforth to use the GeV as a unit of energy and mass; the rest energy or mass of the proton is 0.94 GeV.) In the standard model, there is just one new particle, the “Higgs boson” still to be found, in which case there could then be a “desert”, devoid of new phenomena, extending to the grand unified scale of 10^{14} GeV or beyond. However, there are compelling arguments to expect that, rather than a desert, a fertile region rich in new phenomena awaits exploration.

Accelerators and colliders

The main tool of particle physics is the accelerator. High energy particles are needed both because they have the small quantum

mechanical wavelengths required to resolve the structure of matter on a small scale (the principle of the electron microscope) and also to create heavy particles such as the W and Z, using $mc^2 = E$. When a beam of particles strikes a stationary target, momentum conservation requires the debris to be thrown forward. The kinetic energy associated with this motion is wasted; it cannot be used for creating new particles or probing short distances. Indeed, because of relativity, the fraction of the incident energy which is wasted increases dramatically with increasing energy (the useful energy grows only as the square root of the beam energy). This waste can be avoided by colliding two beams of particles head-on, as in the CERN proton-antiproton collider used to discover the W and Z. To produce the same useful energy with a stationary target, a beam with energy 300 times the combined energy of the proton and antiproton beams would have been required.

Because of this effect, most future experiments will involve colliders. The price to be paid is that a second beam provides a much less dense target than a stationary piece of matter, and thus a much lower collision rate. Furthermore, only stable charged particles (protons (p), antiprotons ($\bar{p}$), electrons (e) and antielectrons or positrons ($\bar{e}$)) can be stored and collided, although this is not a serious limitation in addressing fundamental questions because all combination of lepton, quark and gluon interactions can be studied with pp , $p\bar{p}$, $e\bar{e}$ or ep colliders.

The characteristics of some of the world's front-line machines, now running or under construction, are shown in the table below. One striking feature of this table is that the proton beam energies are much higher than the electron energies. The explanation is that when charged particles are deflected by the magnets that guide them around circular accelerators, some of the energy stored in the electromagnetic field accompanying the charge carries on in a straight line, generating synchrotron radiation. This radiation may be a bonus for those who can use it as a tool, but it is an expensive problem for particle physicists. To reduce the energy lost by radiation, circular electron machines must be made very large and this proves to be the limiting cost factor. Synchrotron radiation is unimportant for protons because they are so much heavier, which is why they can be accelerated to much higher energy than electrons for a comparable cost.

Nevertheless, electron machines are able to hold their own because, in a proton machine, the energy of the proton is shared among a cluster of quarks and gluons so that an individual quark or gluon typically carries only one tenth to one fifth of the beam energy. Moreover, the interpretation of proton collisions is complicated by the fact the fraction of the energy carried by individual quarks and gluons is not fixed.

The cost of circular electron machines increases very rapidly with energy because of synchrotron radiation. Above about 150 GeV per beam, it will become cheaper to collide beams from two opposed linear accelerators, and the long-term future of $e\bar{e}$ colliders depends on whether this is practicable. A first test will be provided by the SLAC Linear Collider (SLC), which will use an existing linear accelerator to accelerate beams that will be separated and collided. Since the beams will then be thrown away, a reasonable collision rate can be achieved only by focusing them to about one micrometre in diameter. Producing and colliding such beams is a formidable challenge, comparable with that faced in producing and colliding intense bunches of antiprotons at CERN.

The experimental programme

Experiments at colliders are carried out with very large detectors which surround the collision point and are designed to measure the energy, momentum and nature of the particles produced. Every detector is a compromise, and different detectors are designed to have different strengths in their abilities to study different types of particle, to measure energy and so on. Some idea of their typical size is provided by the UA1 detector at the CERN $p\bar{p}$ collider, which is a $10\text{m} \times 10\text{m} \times 10\text{m}$ cube packed with sophisticated equipment. Such instruments are always used for several years, with each period during which the accelerator is run corresponding, in a sense, to a new experiment, because the apparatus is often modified and the way it is operated is changed in the light of previous results. For example, in each run, suitable trigger conditions must be chosen to recognize instantaneously especially interesting categories of events whose characteristics are recorded for later detailed study, because the total number of events is usually far too large for all to be recorded and analysed.

How will future experiments cast light on the grand problems of particle physics? Two caveats apply to what follows. First, inevitably, the discussion must be confined to the framework of existing speculation. Experiments in the next decade will probe the structure of matter at one-tenth of the distance scale explored so far, and it will be surprising if they do not produce surprises. Second, the discussion is concerned only with measurements bearing on really fundamental questions. Future experiments will also allow detailed studies of phenomena whose fundamental character is already known, but about which there is nevertheless still much to be learned.

The discussion will be organized on the basis of theoretical problems rather than machines, but it will become clear that different types of collider ($p\bar{p}$, $e\bar{e}$, ep) have complementary capabilities. Very crudely, $p\bar{p}$ and pp colliders reach the highest energy, but are relatively blunt exploratory instruments compared to $e\bar{e}$ and ep

machines. For example, while pp collisions yield some information about the distribution of quarks and gluons in the proton, it is crude compared to what is learned from "X-raying" the proton in ep collisions. Similarly, although the Z and probably the t quark have been discovered at a $p\bar{p}$ collider, investigation of their detailed properties must await SLC and LEP. Proton-antiproton collisions at CERN have already produced tens of Zs, but electron-positron collisions will resonate at the rest energy of the Z, which may be produced at a rate of tens, or perhaps hundreds, of thousands per year in the SLC experiment and millions per year in each of the four simultaneous experiments planned for LEP. This will allow high precision studies of the Z itself, and of its decay products, including the t quark and any as yet undiscovered particles less massive than the Z. Finally, although existing $p\bar{p}$ colliders have enough energy in principle, only LEP when upgraded to more than 85 GeV per beam will be capable of studying the production of pairs of Ws so allowing crucial tests of the electroweak theory. This is why the LEP design is optimized for eventual running at 100 GeV per beam.

Future experiments will address the "grand questions" in the following ways:

● **Particles.** New charged particles can most easily be found in $e\bar{e}$ collisions, in which an electron and positron annihilate forming an unstable quantum of electromagnetic energy which disintegrates into whatever charged particles it can. From PETRA experiments, we know that there are no charged leptons between the τ , with a mass of 1.7 GeV, and about 20 GeV. SLC will extend the search to 50 GeV, while LEP will eventually explore the region up to 100 GeV. SLC and LEP will make detailed studies of the t quark, if its mass is around 40 GeV as suggested by recent CERN data, and LEP will eventually discover any other quarks with masses less than 100 GeV.

If there are additional neutral leptons (neutrinos) with masses less than half that of Z, it will decay into them. Although such decays cannot be observed directly, they would alter the Z's half-life in a way that could be inferred from experiments at $p\bar{p}$ and $e\bar{e}$ colliders. We have already learned from the CERN $p\bar{p}$ collider that there cannot be more than about five such leptons. If any exist, they will be discovered at SLC or LEP. In addition to the obvious importance for particle physics, knowledge of the number of light neutrinos is vital for astrophysics, for it determines how fast the Universe expanded when it was a few minutes old, which in turn controls the cosmic abundance of helium. The number of neutrinos also influences the fraction of the energy of the Universe which may now be stored in neutrino background radiation, a quantity which is very sensitive to whether neutrinos actually have small masses, as is strongly suggested by GUTs. This question is now being vigorously

Some front-line facilities		
Europe	Existing	Under construction
CERN	SPS 450 GeV p $E_{cm} = 30$ GeV	LEP (1989) 50 GeV $e + 50$ GeV $\bar{e}$ $E_{cm} = 100$ GeV ($E_{cm} \approx 200$ GeV, 1992?)
	SPPS 320 GeV $p + 320$ GeV $\bar{p}$ $E_{cm} = 640$ GeV	
DESY	PETRA 23 GeV $e + 23$ GeV $\bar{e}$ $E_{cm} = 46$ GeV	HERA (1990) 35 GeV $e + 900$ GeV p $E_{cm} = 350$ GeV
USA		
	FNAL Tevatron 800 GeV p $E_{cm} = 40$ GeV	TeV (1986) 1,000 GeV $p + 1,000$ GeV p $E_{cm} = 2,000$ GeV
SLAC		
	PEP 15 GeV $e + 15$ GeV $\bar{e}$ $E_{cm} = 30$ GeV	SLC (1987) 50 GeV $e + 50$ GeV $\bar{e}$ $E_{cm} = 100$ GeV.

The acronyms of each laboratory are listed in the left-hand column. Data given for each machine are: completion date, in parentheses; energy of beam in GeV, type of particle [proton (p), antiproton ($\bar{p}$), electron (e), positron ($\bar{e}$)] + energy of opposing beam and type of particle in the case of colliders. E_{cm} = centre of mass energy; this is the useful energy available for creating new particles. 1 GeV is slightly more than the energy (mc^2) of a proton at rest, which is 0.94 GeV. A machine with $E_{cm} = 50$ GeV can in principle create a particle 50 times as heavy as the proton.

attacked in various relatively low energy particle physics experiments. Some astrophysicists claim that the observed abundance of helium makes it unlikely that there are more than one or two undiscovered light neutrinos. A direct measurement in Z decay, which could also find heavy neutral leptons, is obviously highly desirable.

If quarks and leptons are composite, they may have detectable structure and excited states should exist. According to one school of thought, the W and Z are also composite and share common constituents with quarks and leptons. If so, structure should show up at a distance scale set by the mass of the W that will be probed by experiments at $p\bar{p}$ colliders, at SLC, LEP and HERA, which will, in their different ways, extend the search for structure down to one-tenth of the distances studied hitherto. Enthusiasts for composite models claim that the unexplained events recently observed at the CERN $p\bar{p}$ collider may be manifestations of excited states of quarks.

● **Forces.** Further tests of the unified electroweak theory are badly needed for two reasons. First, it is still conceivable that the W and Z are composite particles, unconnected to the photon. It can be argued that, nevertheless, they might have properties similar to those predicted by the standard model if they are very tightly bound states of constituents with masses of about 1,000 GeV. In that case, however, their properties would be nonstandard to a degree that should be measurable in forthcoming experiments and there would also be heavier particles made of the same constituents. Second, if, as seems much more probable, the standard model is basically correct, it is very likely that it is part of a completely unified theory. If so, there must be heavier W and Z-like particles and the ordinary W and Z might have slightly non-standard properties.

Many new tests of the electroweak theory will become possible in the coming decade. At SLC and LEP, accurate meas-

urements of the mass of the Z and of the cross-section for the production of muon pairs will be particularly important. An upgraded LEP will also be able to study W pair production, which is sensitive to basic untested features of the electroweak theory and may serve to distinguish it from models in which the W is composite. Experiments at an upgraded LEP will also be sensitive to the effects of additional Zs with masses up to about 500 GeV. Experiments at HERA which study electron-quark scattering, and the reaction electron + quark $\rightarrow$ neutrino + quark, will allow independent high-precision tests of the electroweak theory and will be sensitive to the effects of additional charged Ws with masses up to about 500 GeV, which could not be seen at SLC and LEP.

In GUTs, some, perhaps all, of the additional Ws and Zs are supposed to have masses of the order of 10^{14} GeV. Exchange of these very heavy particles is expected to generate proton decay. Since, by definition, leptons do not feel the strong force, while quarks do, it is not surprising that in GUTs, in which there is no fundamental distinction between the strong and the electroweak forces, there is no fundamental distinction between quarks and leptons. Thus GUTs unify quarks and leptons, as well as unifying forces, and GUT forces can transmute quarks into leptons thus allowing the proton to decay.

The prediction that the proton is unstable is exciting first because it alters our view of the permanence of matter, second because it may explain the imbalance between matter and antimatter in the Universe, and, third, because it may be testable. Large detectors have been constructed to examine the stability of large quantities (hundreds or even thousands of tons) of matter in deep mines, shielded from most of the effects of cosmic radiation. These experiments are at an exciting stage, having already reached the sensitivity required to see proton and neutron decays according to some varieties

of GUTs. They have found a few events that defy simple explanation but more results, and perhaps the more sophisticated detectors now being constructed, will be needed to determine the origin of these events. These experiments are also sensitive to any powerful cosmic sources of neutrinos (they might eventually be able to detect neutrinos from supernovae) and to any new highly penetrating particles that might be present in cosmic rays, such as the magnetic monopoles whose existence is predicted by GUTs.

● **Masses.** The known solutions to the problem of why the W and Z are massive all involve new phenomena that may be discovered in forthcoming experiments. The photon, which is believed to be massless, can exist in two independent states, which is why ordinary light waves can be polarized in two independent ways. But "heavy photons" such as the W and Z can exist in three independent states. (Technically the photon has spin one: if it were massive, we could always find a frame of reference in which it was at rest and then orientate the spin in three independent ways.) The question of why the W and Z are massive while the photon is mass-less, although it is their sibling according to the unified electroweak theory, is therefore directly related to the origin and nature of their third states. There are three possibilities:

- (1) The third states of the W and Z may be fundamental particles. This is the case in the textbook formulation of the standard model, which contains fundamental entities called Higgs fields, or particles, which induce a preference for a solution in which the symmetry is hidden. The Higgs particles supply the third states for the W and Z, thus distinguishing them from the photon and enabling them to become massive. However, it turns out, inevitably, that one Higgs particle is left over as a new independent type of particle called the Higgs boson. Its mass is not predicted by the theory, but must be less than a few hundred GeV for the theory to make sense. Searches for this particle are obviously extremely important. Only $e\bar{e}$ colliders provide a reasonable chance of finding it. If its mass is less than 50 GeV, it may be found in high statistics studies of Z decay at LEP. If it is heavier, it may be produced in conjunction with a Z at an upgraded LEP.
- (2) The third states of the W and Z may be composite particles. The hypothetical constituents of these composite particles are usually referred to as "techni-quarks", and the force which is supposed to bind them together as "technicolour". It is difficult to make technicolour models which are compatible with all the data, but they have some very attractive features and must be taken seriously. If the underlying idea is correct, there should be other bound states of the constituents which can be sought at SLC and LEP, and non-standard behaviour may show up in W pair production at an upgraded LEP.
- (3) All three states of the W and Z may be

composite and unrelated to the photon, in which case the mass problem would be transferred to the constituent level. As discussed above, this possibility will be tested in future experiments.

Among these possibilities the "Higgs mechanism" for mass generation is the most conservative in the sense that it predicts the existence of only one additional undiscovered particle. However, the masses of the W and Z introduced by the Higgs mechanism are unstable, and tend to be dragged up to the largest mass scale available, presumably the 10^{19} GeV scale associated with quantum gravity. In the standard model, they can be held down at their observed values, of about 100 GeV, only by a highly artificial balancing of the parameters in the theory, which must be "tuned" to an accuracy of one part in 10^{26} . This instability can be avoided only by introducing a new hidden symmetry, called supersymmetry (SUSY). SUSY is appealing for many reasons. It unites particles with different quantum mechanical spins in common families. It provides a natural framework for unifying gravity with other forces, since when SUSY is made local, in the sense of local gauge invariance, it turns out to be part of a theory that includes Einstein's theory of gravity. Finally, according to rather general theorems, SUSY is the only type of symmetry possible for which there is not already some experimental evidence. It would seem parsimonious of nature to have exploited all symmetries but one, so we should take the possibility of an underlying supersymmetry very seriously.

According to SUSY, every particle should have a "superpartner" whose quantum mechanical spin differs by half a unit. Thus, corresponding to the known quarks and leptons which have spin one-half, there should be spinless squarks and leptons, known in the trade as squarks and sleptons, and there should be spin one-half partners of the W, the Z, the photon and the gluon, which have spin one. It turns out that, in order to avoid the instability that led us to SUSY, these superparticles must lie below 1,000 GeV in mass and, in most models, some of them have masses similar to that of the W. If this is so, there is a good chance that they will be found in the next generation of experiments. Experiments at the $p\bar{p}$ collider may discover squarks and spin one-half gluons provided they are not much heavier than 50 GeV. Enthusiasts for SUSY claim that the recent unexpected results found by these experiments may already provide evidence for superparticles. Experiments at HERA may discover some particular squarks and sleptons with masses up to about 150 GeV, the exact limit being model-dependent. SLC or LEP will find any charged superparticles, and quite probably also neutral superparticles, whose masses are less than 50 GeV. Upgraded LEP will extend the search to 100 GeV. If superparticles exist, $e\bar{e}$ experiments will provide the best way to

study their properties in detail.

Conclusions

There has been remarkable progress in particle physics in the past two decades. Our description of the observed particles has been greatly simplified by the discovery of an underlying quark substructure. Following the triumph of the gauge principle, it is perhaps not too much to claim that we now know the origin of force. This progress has been synthesized in the so-called standard model, which successfully describes a vast amount of data, but it has been recognized from the beginning that this model cannot be complete. Theorists have been searching energetically for more complete models, but there are too many options and the baton is now in the hands of our experimental colleagues.

The present experimental situation is exciting. Experiments at the CERN $p\bar{p}$ collider and proton decay experiments have recently given hints of phenomena that may lie outside the framework of the standard model.

With the Fermilab $p\bar{p}$ collider, SLC, LEP, and HERA under construction, there is a rich experimental programme ahead that will address many fundamental questions. Experiments in the next decade will extend our knowledge of the spectrum of quarks, leptons and any other particles to much higher masses. They will test the unified theories of forces. They may reveal the origin of the masses of the W and Z, which seems to be intimately linked with the origin of all masses. They will explore uncharted energy and distance scales and may reveal phenomena as yet undreamt of.

Looking beyond the machines now under construction, it is already clear that a 12,000 GeV proton collider could be built in the LEP tunnel at CERN for much the same cost as the LEP project, and energies up to 20,000 GeV may become possible; as well as pp collisions, such a machine could generate ep collisions at six or more times the HERA energy, since one beam could be collided with an electron beam in LEP. Scientists in the United States are considering a more ambitious 40,000 GeV proton Superconducting Super Collider (SSC), which would be far more expensive because it would be much larger and would require the construction of a new tunnel. Indeed, construction of the SSC would require the annual US particle physics budget to be doubled, but even this increase would only restore expenditure to the same fraction of GNP as in the mid-1960s. It is therefore possible to imagine an experimental programme stretching a quarter of a century ahead, if it is scientifically desirable. In the more distant future, novel techniques will have to be found to contain the cost of accelerators if it proves imperative to go to even higher energies. □

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Nuclear winter and carbon dioxide

The National Academy of Sciences has at last reported on the nuclear winter. It clarifies the scientific uncertainties that affect other important climatic models.

THIS week's report on nuclear winter by the US National Academy of Sciences may be late (by more than a year), but is nonetheless welcome. The document, prepared by a committee under Dr Georges Carrier of Harvard University, was a key reference in the paper that remains the most coherent account of what the nuclear winter would be like, that of Turco *et al.* (*Science* **222**, 1283–1292; 1983). Much of the delay has come about because of the diligence of referees of earlier drafts.

The nuclear winter is the phenomenon by which, in the aftermath of nuclear war, the upper atmosphere will be filled with particles of smoke from fires burning on the surface of the Earth. After a few days, the smoke will be distributed uniformly. If there is enough of it, the Sun will be obscured, the lower atmosphere cooled and a temperature inversion formed that may be stable for weeks or even months. Given persistence, and enough obscuration, surface temperatures would be so low for so long that most living things would die.

During the past year, the concept of nuclear winter has been widely canvassed. Apart from successors to the paper by Turco *et al.*, there have been public meetings, popular articles (of which the first seems to have been that by Carl Sagan of Cornell University in the supermarket magazine *Parade* for November 1983), and several speeches by politicians. Under the auspices of the International Council of Scientific Unions, the Scientific Committee on Problems of the Environment is well launched on a formal study of the problem that is due to be published next year.

It is in no sense surprising that the issue has been controversial. Most issues concerning nuclear war have this effect. Earlier reports on the consequences of nuclear war by the National Academy of Sciences, most recently in 1978, were deficient in their treatment of the consequences of atmospheric soot, a circumstance first pointed out by P.J. Crutzen and J.W. Birks (*Ambio* **11**, 114–125; 1983). I have run into trouble with Turco *et al.* for suggesting, on grounds disputed, that their original article had been "hyped", as publishers say (see *Nature* **307**, 107; **308**, 11 & **311**, 307; 1984), but I had not known then that the launching meeting had been supported by a grant of more than \$50,000 from the Boston-based Kendall Foundation to a public relations firm. Edward Teller made a similar case more

convincingly (see *Nature* **310**, 621; 1984), if at greater length.

The National Academy's new report is a major contribution to this evolving discussion. (A full report of it will appear in *Nature* next week.) Inevitably, its effect is to accept that nuclear winter could be a consequence of a major nuclear exchange but to emphasize more clearly than has been customary the uncertainties in detailed calculations of what a nuclear winter would be like or how long it would last. Nobody will be surprised. In any attempt to predict the behaviour of a system as complicated as the atmosphere, it is natural that the starkness of first approximations should be relieved when refinements are introduced.

Those who think otherwise could do worse than glance at almost any issue of the purple version of the *Journal of Geophysical Research*. The October issue, for example, contains an account of one of the most ambitious attempts to calculate the climatic consequences of increased carbon dioxide in the atmosphere (Washington, W.M. and Meehl, G.A. *J. geophys. Res.* **89**, 9475–9503; 1979). The authors have used the versatile community climate model they have helped to develop at the National Atmospheric Research Center (NCAR).

The calculation is both an illustration of how competent the climate modellers have become and of how far they have to go. The authors have set out to calculate the properties of two atmospheres, one with the present concentration of carbon dioxide, one with twice as much. The surface of the Earth is given a "realistic geography", which means that the continents are accurately placed where they should be, so that the variation of albedo over the surface of the Earth is not very different from reality. But the oceans are represented only by a slab of water 50 metres thick, which allows for seasonal heat exchange but not for global heat transport, a simplification which is sensible enough when the objective, as here, is simply to calculate the equilibrium condition of two atmospheres containing different amounts of carbon dioxide.

This version of the model is persuasive. It reproduces well enough the seasonal variation of the atmosphere as it is. Stratospheric temperatures come out well, for example, as do the general patterns of surface temperature and even wind velocity. Precipitation is less well calcu-

lated, sea-ice cover is over-estimated (perhaps because of the neglect of global oceanic transport), as are summer continental temperatures (because of the modellers' endless problem of allowing accurately for clouds).

The prediction of what the climate would be like if the carbon dioxide were twice as abundant as at present is in the circumstances all the more telling. Briefly, the greatest differences of temperature are at the poles, but also at mid-latitudes, while precipitation is everywhere increased. Inevitably, to the modellers, calculation has been seriously constrained by the speed with which even NCAR's computing resources can handle climatic fluctuations from one season to another. And this, in a sense, is merely an equilibrium calculation. Rapidly changing systems should be inherently more difficult to calculate accurately, although it may then be possible to omit some slowly-changing components of the climatic model.

Schneider *et al.* have shown (*Nature* **308**, 21; 1984) that a different version of the community climate model can indeed be used to simulate nuclear winter; they decided at the outset to take the properties of the atmospheric soot layer as given. But that, of course, is where much of the argument about nuclear winter centres. Will the layer of soot be uniform and, if not, will it be stable? How long, in any case, will it last? To ask these questions is not to suggest that the nuclear winter is a kind of hoax but rather that the nagging difficulties in the original account persist — the National Academy's report clarifies but does not answer them.

For what it is worth, the same conclusion seems to apply even to the more fully studied problem of carbon dioxide. The same issue of the same journal gives an account of accurate measurements carried out in the past decade at three Canadian stations of the atmospheric concentration of carbon dioxide. The detail is absorbing, but C.S. Wong *et al.* (*J. geophys. Res.* **89**, 9527; 1984) also confirm what others have recently suggested, that the rate of increase of carbon dioxide (at 1.4 parts per million per year) is between a third and a half of the rate measured in earlier decades. The reasons for this change are entirely unknown, but presumably involve interactions with either the sea or the biosphere. Washington and Meehl's work is no doubt still valid, but it will be longer before it comes into its own.

John Maddox

Magnetosphere

First success for a space mission and a comet for Christmas

from D.J. Southwood and D.A. Bryant

THE first active phase of the Active Magnetospheric Particle Tracer Explorers (AMPTE) space mission is successfully accomplished. On 11 September 1984 and again on 20 September, lithium ions were released in the solar wind immediately upstream from the terrestrial magnetosphere, some 110,000 km from the Earth's surface. At a meeting of the AMPTE science team on 25–27 October at the Applied Physics Laboratory in Laurel, Maryland, the data from the releases were assimilated and cross comparisons were made between the three spacecraft involved in the mission.

AMPTE is a joint project of the United States, West Germany and Britain. Each national team has provided a spacecraft. The US Charge Composition Explorer (CCE) carries a comprehensive set of instrumentation to detect the mass and charge composition of the terrestrial ionized environment, the magnetosphere. Its task is to monitor whether test ions released by the German Ion Release Module (IRM) can penetrate to its orbit. The IRM is also fully instrumented to make plasma and field measurements. The third spacecraft is the British subsatellite, UKS, added to the programme at the behest of the German team, who recognized that the actual pick-up of the release ions by the surrounding plasma posed a significant scientific problem in its own right. The UKS orbits close to the IRM so that both spacecraft are able to monitor the local effects of the ion releases.

The first result of the release experiment in the solar wind is a null one; the team are convinced that no lithium ions made their way from the release site to the CCE, deep in the magnetosphere. However, no disappointment was evident in the team, in part because of their exhilaration at the unique measurements made by IRM and UKS as the ions were created. In any case, the null result provides an important constraint on our understanding of solar ion access. Furthermore, the quality of the data from the passive measurements made routinely in the mission is so good that inevitably major gains will be made in our knowledge of the Earth's ionized environment and in its interaction with the solar wind.

The lithium releases produced artificial cometary effects. Comparison of IRM and UKS data show that the clouds initially formed a cavity in the solar wind from which the magnetic field was excluded. The scale of the cavity must have been well constrained because in both cases it contained IRM, whereas UKS saw no cavity in one case, and in the other only partial exclusion

of the field. The solar wind was slowed and diverted by the obstacle; in the process, the solar wind magnetic field was amplified. Magnetic forces led to the cloud being blown away from the vicinity of the spacecraft. Electron heating accompanied the field compression and the ion detectors recorded the signatures of classic cycloidal motion of the lithium ions as the solar wind picked them up and accelerated them. Further detailed plasma diagnostics were provided by plasma wave instruments. Beam instabilities, attributable to the interaction of lithium ions and solar wind protons, were detected but, during the passage over the spacecraft of the most intense electrical current sheets, no 'anomalous' (wave-induced) electrical resistivity seems to have been present.

Between the active ion-releasing phases of the mission, the three spacecraft make passive measurements of the Earth's ionized outer atmosphere. The measurements made during the magnetic storm of 4 September provide a high point of the

mission so far; CCE monitored the build up and decay of a hot plasma 'ring current' about the Earth, and IRM and UKS detected extreme solar wind, bow shock and magnetopause conditions.

The next ion release from IRM is due to take place on 25 December at 12.18 UT. The date was chosen for scientific reasons; the ideal configuration of the spacecraft and the ambient flow, combined with the requirements imposed by the position of viewing sites, constrained the team to two dates, of which Christmas Day is preferred to 27 December. The release will be of barium, which ionizes much faster than lithium, and will be made on the dawn flank of the magnetosphere, just outside the magnetopause. A longer-lasting and more substantial cavity is expected. The prime purpose is to create an artificial 'comet' which will be visible from North America and the eastern Pacific. The comet may be visible to ground observers (and other wise men) on the dawn side of earth for about twenty minutes.

Further releases of barium and lithium are planned for late March 1985. The ions will be released into the tail of the magnetosphere and CCE will be tracing the access of the ions to regions closer to Earth. □

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Gene regulation

Repression of activators

from Anna Velcich and Edward Ziff

SINCE their first discovery in 1981, gene enhancers have increasingly been recognized as potent activators of transcription of a wide range of viral and cellular genes¹. A challenging report by Borrelli, Hen and Chambon on page 608 of this issue presents evidence of a mechanism of negative regulation of a viral enhancer². The finding is even more provocative because the proteins that Borrelli *et al.* show to repress enhancer activity are best known for their ability to activate genes. They are the E1a proteins of adenovirus-2, which are known to stimulate the transcription of both adenoviral and cellular genes in cells infected by the virus.

Enhancers are short segments of DNA that indirectly stimulate the transcription of nearby genes, perhaps by providing access for the transcribing enzyme, RNA polymerase II, to the genes. By contrast, the E1a proteins are direct activators of gene transcription^{3,4} by mechanisms that are unknown.

Chambon and his colleagues have studied the effect of E1a proteins on transcription of a plasmid containing a rabbit β -globin gene linked to the enhancer of polyoma virus. They show that when HeLa cells are transfected with this plasmid, the

polyoma enhancer stimulates transcription of the β -globin gene, but transcription is repressed if the cells are co-transfected with a plasmid that expresses E1a proteins. Unlike activation, the repression is independently effected by either of the E1a proteins. In competition experiments, additional enhancer DNA relieves repression by E1a, presumably by directly and competitively binding repressor. The E1a gene's own enhancer is active in this competition assay, suggesting that it may itself be repressed by E1a proteins and so be autoregulated. Chambon *et al.* show that the enhancer of simian virus 40 too is a target of repression, a result that is also indicated by data from our laboratory and which suggests that the repression of enhancers by E1a could be quite general.

Because, as the data show, activation and repression can be exerted under the same conditions, the E1a gene may command a complex control over transcription. With increasing production of E1a protein, a gene stimulated by it would be expressed and a gene dependent upon enhancers would be repressed. As the quantity of E1a protein falls, the activity of the E1a-dependent gene would dwindle, and the enhancer would regain its activity. The

activities of the two genes would be inversely related, with E1a providing a sort of 'flip-flop' control.

The focus on E1a proteins is of particular relevance because they are known to exert a profound effect on cellular metabolism, activating chromosomal genes for both heat shock protein⁵ and β -tubulin proteins⁶. E1a proteins have also been shown to activate certain other cellular genes in recombinant plasmids^{7,8}. In addition, as first reported by Houweling *et al.*⁹, if primary rodent cells are transfected with cloned E1a genes alone, they become immortalized. The mechanism of immortalization is unknown but may be the result of stimulation by E1a proteins of the same cellular proliferative functions that are stimulated by the proteins for the benefit of the adenovirus in infected cells. Perhaps the E1a proteins are mimicking the action of an unidentified endogenous stimulator of cell growth that is under the strict control of extracellular growth regulators.

Since the report of amino acid sequence homology between the E1a and myc oncogene proteins¹⁰ and the finding that either the E1a gene or the myc gene will complement a *ras* oncogene in transforming primary cells^{11,12}, any newly discovered property of myc or E1a is promptly sought in the other. Current evidence correlates myc gene expression with cellular proliferation, a feature perhaps akin to E1a's immortalization function. Myc production is transiently induced when growth factors stimulate resting fibroblasts or T cells to enter the G₁ growth state¹³⁻¹⁵. Regulation of the quantity of myc in the cell may also be important to differentiation. Many cell lineages maintain the ability to proliferate until they acquire markers specific for terminal differentiation, whereupon they withdraw from the cell cycle. During *in vitro* differentiation of the HL60 promyelocytic cell line, the expression of myc declines as the capacity for unlimited cell division is lost and differentiation markers are induced (ref. 16 and refs. therein).

Is there evidence for normal cellular proteins with the properties attributed here to E1a? Once again, a virus gives some useful information. The polyoma virus enhancer, shown by Borrelli *et al.* to be blocked by E1a, is inactive in undifferentiated embryonal carcinoma (EC) cells, but becomes active upon their differentiation (ref. 17 and refs. therein). Perhaps a protein in the undifferentiated cells represses the polyoma enhancer, as does E1a in HeLa cells, and perhaps this protein declines in quantity upon cell differentiation. Further evidence comes from the observation that class I major histocompatibility antigens are absent from undifferentiated EC cells but appear upon differentiation (ref. 18 and refs. therein). Provocatively, the E1a gene of the highly oncogenic strain of adenovirus, adenovirus-12, but not that of adenovirus-2, represses these antigens in transformed rodent cells¹⁹ and Borrelli *et al.* suggest that

their expression relies on an enhancer, which could be the target for adenovirus-12 E1a. Finally, EC cells do seem to have an E1a-like protein, which is indeed lost upon differentiation²⁰.

It is tempting to speculate that cellular proteins possessing activator and repressor functions analogous to those of E1a play a role in gene regulation during the growth and differentiation of cells. By inducing a high level of the proteins, a cell might turn on genes required for cell-cycling and repress other genes that are incompatible with proliferation but required in the resting state or after terminal differentiation. Could myc or a related protein provide such regulation? Experiments assessing any role for myc in gene transcription are scant, but there is a hint that, like E1a, myc can activate heat-shock genes²¹. And, as expected for an E1a-like protein, myc does decrease during EC cell differentiation¹⁴. Little is known about enhancer control, but the existence of tissue-specific enhancers^{22,23} makes it highly probable that there is regulation during differentiation. E1a's intriguing properties are directing us to fundamental features of the control of genes and, with time, all will be revealed. □

Low-temperature physics

Do cosmic rays account for superfluid ³He transition?

from P. V. E. McClintock

IN THEORY, the transition between the two principal superfluid forms of liquid ³He ought not to be able to occur. So why is it routinely observed to take place? A possible answer to this intriguing question has recently been put forward by A. J. Leggett. Writing in *Physical Review Letters* 53, 1096; 1984, he suggests that the transition, although prohibited from occurring spontaneously, readily takes place because of the occasional high energy cosmic ray that passes through any experimental chamber on the Earth's surface.

When helium gas is sufficiently cooled under atmospheric pressure, it eventually — albeit somewhat reluctantly on account of its very weak interatomic forces — condenses to form a colourless liquid. The liquid is of exceptional importance and scientific interest, enjoying the unique distinction of remaining a liquid down to the lowest attainable temperatures. There are two distinct forms of liquid helium, corresponding to the two stable isotopes, ³He and ⁴He. Liquid ⁴He undergoes a superfluid transition at about 2 K, entering a state in which a component of the liquid loses all its viscosity. It thus becomes able to flow quite effortlessly through holes or pores of vanishingly small dimensions; correspondingly, a small object travelling through the superfluid encounters absolutely no resistance to its forward motion. It

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requires temperatures about 1,000 times colder for the same sort of behaviour, but in much more complicated form, to be observed in liquid ³He.

When liquid ³He is cooled under a pressure of more than about 20 atmospheres in a weak magnetic field, there is a very rapid onset of superfluidity at about 2 mK. The transition is second order, with no associated latent heat, and it always occurs at the same characteristic temperature for any given pressure. The superfluid phase that results is known as the A-phase. If the liquid is cooled further, it subsequently undergoes a first order transition, to the so-called B-phase superfluid. This transition is analogous to the more familiar phase changes of boiling or freezing, and occurs at a temperature that is not accurately reproducible. To put it another way, if the liquid is being cooled at a steady rate, the time taken for transition from A to B to occur is different on each occasion even if each experiment is carried out with the same cell and, so far as can be determined, in an identical fashion.

There is worse to come. In any pure system close to a phase transition, one can imagine small regions of opposite phase continually forming and disappearing again as the result of thermal fluctuations. Whether any one such region subsequently grows or shrinks will depend both on its

size and on the various bulk and surface energies of the two phases. Thus, $^3\text{He-A}$ could be cooled to a temperature where it would 'like' to become $^3\text{He-B}$, because the energy of the latter phase is lower, but cannot until a spontaneous thermal fluctuation produces a 'large enough' region of $^3\text{He-B}$. Large enough, in this context, means that the increase in the surface energy between the two phases, resulting from a small increase in the radius of the bubble of B-phase, is more than balanced by the corresponding decrease in energy due to the enclosed liquid being in the lower energy state: if this criterion is met, then the bubble of B-phase will obviously tend to expand until it has taken over the whole of the sample, whereupon the transition from A to B will be seen to have occurred.

It is possible to calculate the critical radius for the bubble of $^3\text{He-B}$ and hence, by the application of statistical mechanics, to estimate the average length of time before the transition occurs, given any particular degree of supercooling. Even on the most optimistic assumptions about the numerical values of the various parameters, the calculated result is a period of time vastly in excess of the age of the Universe. Hence, the transition should never be observed. And yet, even with a moderate degree of supercooling, the observed transition time is of the order of minutes.

Leggett's solution to this apparent impasse is based upon a consideration of the events that should follow the transit of a cosmic ray muon of about 2 GeV through the supercooled liquid, as can be expected every few minutes. The immediate consequences are reasonably predictable on the basis of detailed studies of high energy particles in helium bubble chambers: a number of relatively low energy electrons would be produced, each of which would give up much of its kinetic energy through the production of heat, initially creating a region of a few hundred Å in radius at a few tenths of a K, while the bulk of the liquid remained at its ambient temperature of 1–2 mK. What would happen next is a matter of some conjecture. Leggett's view is that an expanding shell of heated liquid would be created, propagating out from each original event, with the enclosed liquid cooling towards its ambient temperature. He points out that this 'baked Alaska' distribution would provide ideal conditions for the formation of B-phase ^3He as the heated liquid cools through the superfluid transition, and that the cooling would be too rapid for the expansion and domination of any small bubbles of A-phase ^3He that might be nucleated at the same time. From the point of view of the experimenter, unaware of the passage of the initiating cosmic ray, the transition from A to B will seem to have taken place spontaneously, a few minutes after the regime of a supercooled A-phase has been entered.

Now that Leggett's suggestion has been

aired, it should not be too difficult to set up suitable particle detectors and a coincidence counting system to test whether the transition coincides with the passage of a cosmic ray through the liquid. If the hypothesis is correct, it will open the way to study the metastable A-phase right down to near-zero temperatures in weak magnetic fields — a matter of considerable experimental and theoretical interest. All

that should be necessary is to shield the sample from cosmic rays, perhaps by conducting the experiments at the bottom of a deep mine. The first experimental test of Leggett's hypothesis will be awaited with very considerable interest. □

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Oncogenic intelligence

Cell immortalization and transformation by the *p53* gene

from D.P. Lane

A DIRECT role for the so-called *p53* gene of cells in the process of oncogenesis is suggested by three papers in this issue of *Nature*. Parada *et al.*¹ and Eliyahu *et al.*² both show that the protein encoded by the *p53* gene can complement activated *ras* genes in the transformation of primary rodent cells. The third report, from Jenkins *et al.*³ establishes that the *p53* gene can also immortalize such cells.

The *p53* protein was first detected in the form of its tight non-covalent complex with the large T protein of simian virus 40 (SV40)⁴. Subsequently it was found also to complex to the E1b 58K protein of adenovirus⁵. Though not closely related in structure, both these proteins are involved in the oncogenic action of their viruses, implying that their shared ability to bind the *p53* protein is important for their action in cellular transformation. The *p53* protein in primary cells and established non-transformed cells has a very short half-life and is present in minute concentrations, with each cell containing only a few hundred molecules. When bound to either viral protein, the half-life of *p53* is greatly extended and the protein accumulates, reaching concentrations of the order of 10,000 molecules per cell. Many transformed cell lines and primary tumour isolates also contain elevated levels of the protein^{6,7}. Microinjection of antibodies to *p53* into the cell nucleus of normal quiescent cells prevents their stimulation by serum, implying an important natural role for the protein⁸.

The new discoveries are provocative because they suggest that the alterations in *p53* levels and stability in many mouse and human tumours may be directly involved in their altered growth. Parada *et al.* introduced a cloned *p53* gene (linked to the murine leukaemia virus LTR) into primary rat embryo fibroblasts (REF) and Rat-1 cells. While the *p53* gene alone failed to transform either cell type, when it was introduced together with an activated *ras* gene, foci were produced in the REF cultures. Since the *ras* gene alone was also unable to induce foci, the *p53* gene seems to provide a complementation function in this

assay in much the same way as the cellular *myc* gene, adenovirus E1a gene and the large T gene of polyoma virus^{9,10}. Parada *et al.* further establish that cells transfected with both *p53* and *myc* genes give rise to tumours in nude mice. In both the focus-forming assay and the tumorigenicity test, the *p53* gene appears to be less efficient than the *myc* gene.

Eliyahu *et al.* obtain broadly similar results with either REF cells or Chinese hamster embryo fibroblasts. Again, *p53*, like *myc*, complements an activated *ras* gene in focus-forming assays. Interestingly, this group had some difficulty in establishing cell lines from the foci resulting from introduction of *p53* and *ras* genes together, and obtained evidence to suggest that overproduction of the *p53* protein can be very toxic to the cells.

Jenkins *et al.* go on to prove that *p53* can immortalize cells, thus increasing the strength of the similarity between *p53*, *myc* and E1a. The Wistar adult xiphisternum chondrocyte cells they used have a doubling-time of greater than 60 hours and undergo 30 doublings in culture before senescence and cessation of growth. But after transfection with a plasmid that contained the *p53* gene, early passage cultures of the cells had an extended life (200 doublings so far), a shorter doubling-time in serum, and the ability to be transformed efficiently into tumorigenic cells by an activated *ras* gene.

Since *p53* regulation is altered in so many human tumours, an obvious first priority, now is to look for alterations in the *p53* gene in human neoplasia. But deeper issues can also be addressed. Does SV40 large T have the unusual ability to transform primary cells both readily and directly because it first immortalizes by stabilizing *p53* and then induces morphological transformation by some other *ras*-like activity? If so, why are E1a proteins, rather than the *p53*-stabilizing E1b 58K protein, the immortalizing proteins of adenovirus? Moreover, why do certain small amino-terminal fragments of SV40 large T that are unable to complex *p53*, nevertheless have the ability to immortalize cells¹¹?

It is time to start looking more deeply for common attributes of all the immortalizing genes. Do others, apart from large T and *p53*, interact with each other and proceed through a common pathway? That some sophistication in our outlook is needed is highlighted by the recent characterization of an Abelson virus-transformed cell line, L12, that does not express *p53* because its gene has been inactivated by insertion of a retrovirus-like sequence into its first intron¹². L12 cells grow continuously in culture but, unlike other Abelson virus-transformed cells, will not give rise to lethal tumours in syngenic animals. Instead the tumours regress spontaneously despite reaching considerable size. When a functional *p53* gene is reintroduced into L12 cells, those cells which then express the gene are able to give rise to progressive and lethal tumours. So, in this system, the *p53* gene product seems to act as a tumour progression factor, whose effect is only really detectable in an *in vivo* assay. This should delight rather than mystify, because the

existence of a battery of oncogenes of subtly different effects, gives us a better chance to simplify and categorize the apparent plethora of human neoplastic diseases. □

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Immunology

Uses of chimaeric antibodies

from Alan Munro

ANTIBODIES were discovered through their ability to neutralize bacterial toxins, and for almost one hundred years antitoxin sera from experimental animals have been used to save many lives. For a brief period, it seemed that serum therapy would solve numerous medical problems. But it was soon found that repeated injections of a supposedly innocuous 'foreign' antibody can lead to harmful hypersensitivity reactions. With the availability of monoclonal antibodies, many new applications of serum therapy (for example, see last week's *Nature*; Cobbold *et al.* **312**, 548) would become well worth exploring if only hypersensitivity reactions could be avoided.

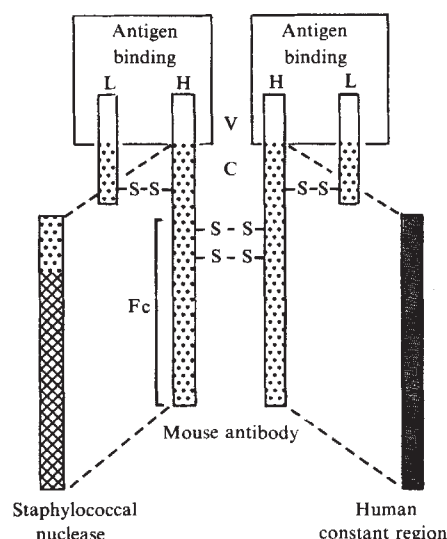
There are three ways to approach this problem. The first is to use monoclonal antibodies from the standard source — hybridomas of rat or mouse cells — but to induce specific unresponsiveness to rodent immunoglobulin before or during therapy. The second approach is to use human monoclonal antibodies, produced either from human hybridomas or from Epstein-Barr virus-immortalized human B lymphocyte cell lines; despite considerable efforts, the success of this approach has been limited and there is no generalized way of obtaining human monoclonal antibodies of the right specificity. Finally, by using the techniques of genetic engineering, it may be possible to obtain antibodies in which the antigen-binding site is defined by sequences from a rodent monoclonal antibody of the right specificity whereas the rest of the molecule is as 'human' in structure as possible.

Papers by G.L. Boulianne, N. Hozumi and M.J. Shulman on page 643 of this issue of *Nature* and by S.L. Morrison *et al.* in *Proc. natn. Acad. Sci. U.S.A.* **81**, 6851; 1984 are the first to describe functional chimaeric antibody molecules containing light chains and heavy chains, in which the whole of the variable regions of the chains are 'mouse' and the constant regions are 'human'. Possibly the human immune system will not recognize such chimaeric molecules as foreign, but there are good reasons to think that it will. In many cases it is possible to mount an immune response against antibody molecules themselves, even when the only antigenic determinants lie in the variable regions (take, for example, the heavy-chain variable region allotypes in rabbits). At least it may be easier to induce unresponsiveness to rodent variable regions when they are part of chimaeric antibodies. In that respect, it will be interesting to compare the rates of clearance of chimaeric molecules with those of the normal antibodies molecules of the host. Even if it proves possible to construct chimaeric antibodies with rodent hypervariable regions embedded in a human variable region framework, it is likely that they will be recognized by the human immune system — but the hope is that such chimaeras will be sufficiently 'self-like' to become integrated into the network of interacting idiotypes and anti-idiotypes without sensitizing the host and so avoiding subsequent hypersensitivity reactions.

The construction of chimaeric antibodies has been made possible through the

development, by a number of independent groups, of vector systems that allow the expression of antibody light-chain or heavy-chain genes in cells that have been transfected with them. Many laboratories have since initiated experiments in which cells are transfected with modified antibody genes. Earlier this year J. Sharon *et al.* (*Nature* **309**, 364; 1984) reported the expression of a novel fragment of antibody consisting of a normal light chain ($V_L C_L$) associated with a modified antibody chain made up of a heavy-chain variable region linked to a light-chain constant region ($V_H C_L$). This fragment binds antigen and is secreted from the transfected cells (showing that the Fc portion of antibody is not necessary for secretion).

The report by M.S. Neuberger, G.T. Williams and R.O. Fox on page 604 of this issue not only confirms that result but goes much further. It shows that the Fc portion of the antibody heavy chain can be replaced by a completely unrelated protein — the enzyme staphylococcal nuclease for example — to produce a bifunctional



Schematic diagram of a mouse antibody showing two identical light chains (L) and two identical heavy chains (H). Each chain consists of variable (V) and constant (C) domains. The antibody class is determined by the heavy-chain constant region which imparts distinctive features on the tail (Fc) region. Neuberger *et al.* have substituted staphylococcal nuclease for the Fc region of both heavy chains of immunoglobulin M; Boulianne *et al.* have replaced the C domain of each heavy chain of the immunoglobulin M monomer with a human C domain.

chimaeric molecule — in this case, exhibiting both specific antigen-binding and nuclease activity. There are many potential applications of this technology in the purification of cloned gene products, in enzyme-linked immunoassays and in the targeting of chemotherapeutic drugs on to tumour cells. □

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Cosmology

Strings and the origins of galaxies

from Neil Turok and David N. Schramm

THE idea that superheavy 'strings', formed at a phase transition in the very early Universe¹⁻⁴, provide an explanation of the origin of galaxies, has been the subject of much discussion (in part in these columns: see *Nature* 310, 365; 1984). We should like to take the discussion further as it is now becoming apparent that strings can make galaxies in ways quite different from other methods and thus may help solve or remove the dark-matter problems.

Strings are predicted to be formed at symmetry-breaking phase transitions in the very early Universe by those grand unified theories (GUTs) in which the vacuum has the appropriate topology^{5,6}. They have well-known analogues in condensed matter physics: for example, the flux lines formed in superconductors. Much as line defects in a crystal, they would form a network across the whole space. The relevant GUTs predict that strings would be formed at a temperature of about 10^{15} GeV at a cosmological time of about 10^{-35} seconds and with a mass per unit length of about 10^{20} kg⁻¹.

In some theories, strings can be formed after a period of inflation. They would be stretched by the subsequent expansion of the universe, and waves on a given scale would begin to oscillate as that scale entered the particle horizon. Whenever the string crossed itself, an exchange of partners could occur and produce closed oscillating loops of string with long lifetimes⁷. The gravitational field of these loops would cause matter to accrete around them⁸. Thus these strings could be the primordial density fluctuations needed in the early universe to explain the eventual formation of galaxies⁹. One point we wish to emphasize and show to be of interest is that, in such models, small loops are cut off from lengths of string which in turn are cut off as larger loops, and so on — in other words, the model creates real spatial correlation between density fluctuations of all sizes.

Until the advent of GUTs and inflation, primordial fluctuations were inserted into cosmology in a completely *ad hoc* way. With GUTs, a fluctuation spectrum with equal power on all scales forms naturally. This is thought to be an excellent way to produce the distribution of sizes in the Universe¹⁰. However, in the normal generation and application of such a spectrum, it is assumed that there is no spatial correlation between the large-scale and small-scale clumps. They each have a random probability (random phase) of occurring anywhere. On the other hand, strings still produce the same spectrum of sizes but also enable a correlation of large scales and small scales.

Galaxy formation with various forms

of dark matter has become an extremely active area of study¹¹. From big bang nucleosynthesis¹² it is known that baryons account for less than 15 per cent of the critical cosmological density. Observations of the dynamics of galaxies suggest that the matter clustered with galaxies is less than 40 per cent of critical density and the lower end of the observed range is consistent with the baryon limits. Thus the dynamical dark matter could be baryons; and some of it must be^{12,13}. There are, however, two arguments which drive us to look for other candidates. The first is inflation (see Guth in ref. 11) and the second is the limit on the anisotropy of the 3K background (see Usson and Wilkinson in ref. 11). Inflation is the only known way of explaining several of the otherwise extraordinary initial conditions of the Universe. But, barring fine tuning, inflation requires a critical density for the Universe; thus at least 85 per cent of the Universe cannot be baryons, and more than 60 per cent of the matter of the Universe did not cluster with galaxies. From the current limits on the anisotropy, Vittorio and Silk (in ref. 11) and Bond and Efstathiou (also in ref. 11) argue that the density of matter in the Universe must be greater than the baryonic upper limit.

To make things even more difficult, it has been noted that the spatial correlation function of rich clusters of galaxies has revealed strong clustering on very large scales (up to 150 Mpc)¹⁴. This correlation function is about 18 times stronger than the spatial correlation function for galaxies⁹, while varying as the same power (-1.8) of the spatial separation, r . (The correlation function is the excess probability over random that an object will be found within radius r of another.) It has also been found that the largest scales in the Universe seem to look filamentary, with large voids and large clumps (see Davis in ref. 11).

Different proposals have been put forth to try to solve each problem, but no model solves them all as long as it is assumed that the primordial fluctuation spectrum has random phases. For example, a model based on low mass neutrinos produces both critical density and large-scale structure (filaments, voids and cluster-correlation function) but does not account for the early formation of galaxies¹⁵. Models invoking heavy- or slow-moving particles (GeV mass photinos, gravitinos, axions or planetary-mass black holes) fit the small-scale structure, galaxy-correlation function, formation times and so forth¹⁶, as well as building hierarchically to yield clusters, but do not allow critical density to be reached. Even hybrid models, with both low-mass and high-mass 'inos', run into problems because the low-mass particles smear out

the small-scale structure. Some of us have even resorted to invoking heavy particles that decay to light ones, but this requires a lot of 'fine tuning'; others (see Bardeen in ref. 11) have suggested solutions where most clumps of matter do not shine as galaxies, thus making galaxies poor tracers of matter and negating the relevance of observational astronomy.

A more natural solution may be the non-random phases of string models. Independently, Peebles¹⁷ and we have noted that non-random phases may help. Such a model yields large-scale filaments and voids as the superheavy strings attract galaxies and clusters, and gives strong cluster-cluster correlations. Recent work¹⁸ has also shown that a model based on clustering of galaxies about filaments fits the higher (3- and 4-point) correlation-functions for galaxies as well as the hierarchical clustering paradigm. Such a model also enables density growth in some areas without producing a large universal background anisotropy and so could enable baryons to be the dark matter on galaxy and cluster scales, with non-baryonic stuff being a critical density background.

The degree of random to non-random phases in such models depends on the density of strings in space. In the limit of space being completely filled with strings, the string-picture also gives random phases. Even if string densities are large enough to randomize phases, their mere existence would still alter galaxy-information calculations, because the strings, rather than the matter, would carry the fluctuations, so standard arguments would not apply. For example, even if the fluctuations are carried by random-phased strings, neutrino streaming would no longer damp out small scales and so the low-mass neutrino model would no longer have problems with galaxy-formation times. To get the correlation function for galaxies to be less than that for clusters means that the fluctuations cannot be totally correlated, so the string density must be moderate. Numerical simulations of the motion of strings¹⁹ and of their spectrum at formation²⁰ suggest that the number of isolated loops falling inside the horizon should be only a few percent of the

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number either formed in association with, or subsequently produced by, the self-intersection of lengths of strings; perhaps ≈ 5 per cent of galaxies should be non-isolated. (Note that even an isolated string will behave differently from a random phase initial perturbation and it will be interesting to see how N-body simulations of galaxy formation are affected.)

Recently, there has been discussion of ways to observe cosmic strings using gravitational lensing effects or to establish the part strings may play in active galactic nuclei^{4,21,22}. Out of this may emerge direct

methods for resolving whether or not strings are present in today's Universe. Although much remains to be done to see if strings are relevant to galaxy formation, they provide an interesting supplement to exotic particles and provide cosmologists with a mechanism for producing non-random phases which totally alter traditional procedures of calculation. □

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Palaeontology

Consensus on archosaurs

from Michael J. Benton

OBSCURITY shrouds the origins of the archosaurs, those diverse, scaled and feathered forms that include pterosaurs and dinosaurs, as well as the living crocodiles and the birds. Commonly, each line is traced back into a rag-bag group of early archosaurs of the Triassic period (208–245 Myr) called the thecodontians. The archosaurs are thought to have arisen just before the Triassic, but the origin and nearest relatives of the group have been obscure. Clarity and a radical new consensus on some of the issues were the outcome of the 3rd symposium on Mesozoic Terrestrial Ecosystems, held at Tübingen, FRG from 6–10 September, 1984.

The reason for the remarkable level of agreement in a hitherto obscured area of evolutionary biology was that each contributor applied the techniques of cladistic analysis to the relationships of the basal thecodontians, and only then slotted the later, well-defined groups into the pattern

obtained. Previous analyses had relied upon the assessment of general resemblances and the time sequence of the fossils in an attempt to pinpoint ancestors. The cladistic technique assumes that it is very unlikely (but not impossible) that we shall ever find an ancestor, and it concentrates on identifying nearest relatives — sister-groups — by an analysis of shared derived characters.

The radical new conclusions that were reached at the Tübingen meeting are best explained by reference to the numbered sequence on the summary tree below.

(1) The archosaurs form part of a larger group, the Diapsida, which also includes the living lizards and snakes^{1,2} (for a dissenting view, see ref. 3). Within the diapsids, the nearest sister-groups to the archosaurs are the rhynchosaurs and the prolacertiforms^{1,2} (J. Gauthier, University of California, Berkeley and G. Paul, Johns Hopkins University).

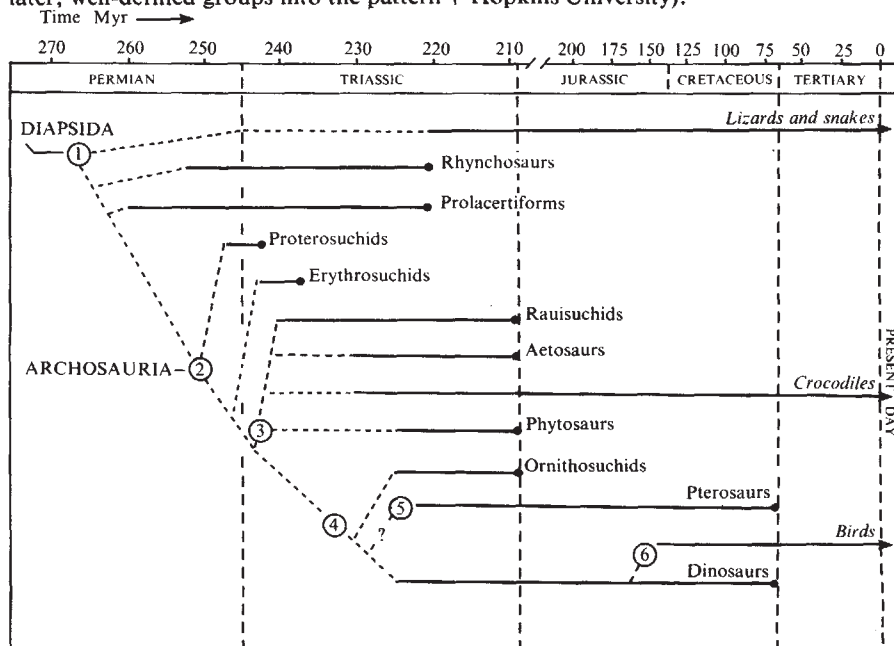
(2) The early Triassic archosaurs — the Proterosuchidae and the Erythrosuchidae — are much more primitive than all later archosaurs, and are placed as basal sister-groups within the Archosauria; the later forms split into two major lines, one leading to the crocodiles, the other to the dinosaurs (M. Parrish, University of Chicago; Gauthier).

(3) The 'crocodile line' includes several specialized thecodontian groups of the middle and late Triassic: the superficially crocodile-like fish-eating phytosaurs, the snub-nosed herbivorous aetosaurs, and the carnivorous rauisuchians. The first crocodiles are known from the late Triassic. They were long-limbed lightly-built terrestrial forms, that evolved aquatic specializations after the extinction of the phytosaurs.

(4) The dinosaurs, as we currently understand that term, form a monophyletic group — that is, they all evolved from a single common ancestor, which was a late Triassic dinosaur. This important conclusion was agreed by Gauthier, Paul⁵ and Parrish, as well as by D. Norman (University of Oxford), P. Sereno (American Museum of Natural History), and myself. Already in the 1970s, several palaeontologists had suggested that the dinosaurs were a monophyletic group^{4,5}, but this view had not been widely accepted. The new consensus is that the dinosaurs were a single group of advanced archosaurs sharing a number of derived characters of the limbs that were related to the animals' particular kind of upright stance (see *News & Views*, *Nature* 310, 101; 1984).

(5) More controversially, Gauthier and K. Padian (University of California, Berkeley) argued that the pterosaurs — the extinct flying reptiles — were the closest sister-group of the Dinosauria. Pterosaurs had forearms, bodies and heads specialized for flight but their hindlimbs are apparently very dinosaur-like.

(6) Finally, there was much more agreement than seemed possible a year or so ago (see *News & Views*, *Nature* 305, 99; 1983) on the relationships of the birds. This was touched on briefly at the Tübingen meeting, but was discussed in greater depth at a special meeting devoted to the 'early bird' *Archaeopteryx* held in Eichstätt, FRG. Nearly everyone now accepts that the birds arose from the dinosaurs, and from the bipedal carnivorous dinosaurs (the theropods) in particular. There was little support for the alternative view that the birds sprang directly from the Triassic thecodontians, or from a crocodile-like ancestor, and the discussion was more about which theropod family was most closely related to the birds. □



A tree (cladogram) representing the evolution of the archosaurs and based on the consensus of opinion at the Tübingen meeting. The tree is set against a timescale based on the known fossil record. Note the change in time scale at 200 Myr, which gives greatest emphasis to events in the Triassic.

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Geology

So you thought mountain ranges were complicated

from Myrl F. Beck

REMEMBER the time when it was thought that the major features of the Earth's crust were riveted in place as securely as armour plates on a battleship? It took courage to be a tectonic theorist, but life may have been easier for the quadrangle-mapper. In those days, crustal blocks mostly moved up and down, or perhaps a few tens of kilometres laterally at most, so one naturally looked for solutions to local geographical problems in one's own backyard. If granitic debris suddenly appeared in a sedimentary section, it meant uplift of the granitic batholith immediately across the valley. There was no need to complicate life by looking any further.

Even with the advent of early plate-tectonic theory the quadrangle-mapper's task was still much the same; unless his map area spanned a major suture, the several parts of his study area could still be assumed always to have been close together, barring a few highly unusual, and geologically easily recognizable, circumstances. But time has complicated this simple picture. In the past decade, a combination of geology and paleomagnetism has shown that at least one orogenic belt (the North American Cordillera) is a moraine of crustal fragments, many with oceanic affinities, transported intact from points of origin hundreds or thousands of kilometres away. Geological studies showed that many adjacent Cordilleran crustal blocks are too unlike one another in stratigraphy and structural history to have evolved in juxtaposition, and that some of these crustal blocks, or terranes, are wholly exotic to North America. To settle this issue, paleomagnetism has provided dramatic evidence of ultra-long-distance transport of crustal blocks.

This microplate model is familiar to many geologists. Orogenesis and the growth of the continent are held to have been more the result of collision and off-scraping of these prefabricated crustal elements than of subduction of the oceanic plates upon which they rode. The model also holds that, once attached to North America, many of these terranes were disrupted by strike-slip faulting; the resulting fragments are now distributed along the continent's edge, placed there by a process that might be termed tectonic longshore drift. Large rotations about vertical axes also may occur during this stage. Because subduction along the western edge of North America has been north-oblique since at least the late Mesozoic, transport and rotation in the Cordillera seem to have been mostly northward and clockwise, respectively. The scale of the process is

hotly debated; some pieces of the Cordilleran mosaic are as small as a house, but others clearly are at least as large as, say, Vancouver Island.

Nevertheless, it still seems to be true that mountain belts are a product of plate interaction, and that they are built at plate margins, usually at the edge of a continent. It should follow that major events in the tectonic history of mountain belts reflect major changes in the behaviour of plates. It should, therefore, be possible to investigate the nature of orogenic cause-and-effect by observing correlations between important tectonic transitions in a mountain belt (as from overthrust to extensional faulting) and events along the plate margin (as, for instance, a change from rapid to slow convergence).

But a mountain belt is an exceedingly complex recorder. Tectonic events overwrite earlier deformations on the same piece of crust without completely erasing the earlier record — thereby making both records hard to interpret. Also, continental crust in an orogenic belt varies enormously in thickness, age, and physical properties from place to place; it seems hopelessly optimistic to assume that the same orogenic response will always follow a particular plate-tectonic event. The timing of tectonic events is a further problem. Such 'events' last several millions or tens of millions of years, and most are complex and progressive — that is, they involve several geological processes acting simultaneously or in sequence. It is hard to know when an 'event' actually starts or stops. Thus, correlations with plate-tectonic phenomena are bound to be difficult to make, and often will not be particularly convincing.

Still more trouble arises because of the nature of the record of relative plate motions. In the Cordilleran example, three oceanic plates (Farallon, Kula, Pacific) and one continental plate (North America) are involved. According to most plate models, direct Pacific–North America interaction has been a factor in Cordilleran tectonics only for the last 30 Myr or so. The chief culprits have been the Farallon and Kula plates, of which, respectively, very little and nothing remain. We deduce the relative motion histories of these two plates from what we hope are mirror-image anomaly patterns recorded on the Pacific plate — thereby putting ourselves explicitly at the mercy of the symmetrical spreading hypothesis of plate tectonics. Likewise, many models make use of the trends of the Hawaiian Islands and the Emperor Seamount chain to deduce absolute motion of the Pacific plate. Any inaccuracy in the

notion that hotspots are fixed relative to each other thus transfers directly to our plate reconstructions. Finally, it has recently been shown that inaccuracies in specifying stage poles for finite rotations increase with the age of the pole, and may become quite large. This means that some of our Mesozoic reconstructions may be seriously in error.

But where was the Kula–Farallon–North America triple junction during the late Mesozoic and early Tertiary? Since the relative motions of Kula–North America and Farallon–North America were quite different at times, the triple junction ought often to have separated regions of distinctly different tectonic style. It may have moved up and down the coastline rather erratically, however. Perhaps the geology will help us locate the triple junction and thereby improve the plate models. The tectonic consequences of transferring large exotic terranes from the oceanic to the continental crust are poorly known; in particular the effect on plate motions is uncertain.

Whether or not the Cordilleran microplate model can be applied to other mountain belts remains to be seen. For the present, pity the poor quadrangle-mapper. No longer can he assume that the batholith across the valley was there when his sedimentary section began recording its influx of granitic debris; without definite paleomagnetic or geological evidence to the contrary, it might instead have been part of, say, Sumatra. □

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100 years ago

The Edible Mollusca of Great Britain and Ireland. With Recipes for Cooking Them.
By M. S. Lovell.

THE primary object of the author is to call attention to the qualities and merits of many kinds of shell-fish which are as nutritious as others which are generally known, but which are rarely met with in our markets, or are only used locally for food, while the proper modes of cooking them are scarcely known. Accordingly all the known species of edible shell-fish on our coasts are here described in succession. This alone would make the volume of great use at a time when we are going to the uttermost ends of the earth for the sources of our food-supply. But when we add that the writer has collected from the most varying sources a mass of curious lore about shell-fish, their uses, and the mode of catching them in various parts of the globe, their medicinal properties, the popular superstitions about them, &c., it will be perceived that this is much more than a work on natural history *plus* a cookery-book. Of all the many uses of snails, the strangest perhaps is that discovered by the London adulterator. They are much employed, the author assures us, in the manufacture of cream, being bruised in milk and boiled, and a retired milkman pronounced it the most successful imitation known!

From *Nature* 31, 124, 11 December 1884.

Direct observations of the dopant environment in fluorites using EXAFS

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Calcium fluoride (CaF₂), like the important UO₂ phase, is a defect system having the remarkable property that large concentrations of trivalent rare-earth dopants can be accommodated within its structure with no loss of structural integrity. Using extended X-ray absorption fine structure (EXAFS), we have observed the way in which the local structural environment of the impurity alters with the size of the rare-earth ion and can distinguish, by computer lattice simulation studies, between dimer and hexamer configurations for light and heavy dopants, respectively.

THE structural properties of anion-excess CaF₂ have been intensively investigated during the last 20 years. Many of the most important problems in the study of non-stoichiometry are raised by these systems which have simple structures and for which well characterized samples are available. Diffraction has been used previously to study the more heavily defective alkaline earth fluorides but is inherently limited when applied to disordered solids as it provides only average structural information. In contrast, extended X-ray absorption fine structure (EXAFS), which yields information on the local structural environment of individual atom types^{1,2}, is uniquely able to distinguish between the complex defect cluster structures proposed for the phase.

The rare-earth trifluorides are highly soluble in the alkaline-earth fluorides. The fluorite structure, typified by CaF₂, can readily accommodate F interstitials which compensate for the charge due to substitutional trivalent dopants. The structure can be easily visualized as a simple cubic array of anions with half the cube centres occupied by cations; the remaining vacant cubes provide the interstitial sites, as illustrated in Fig. 1a.

For low dopant concentrations (≤ 0.1 mol %), it appears that dopants are isolated and that their charge-compensating anions occupy the adjacent cube-centre sites forming nearest-neighbour pairs³. As the dopant concentration increases, evidence from laser spectroscopy⁴ and neutron diffraction^{5,6} suggests that dopant aggregation occurs, forming clusters with two or more substitutional rare-earth ions and their accompanying interstitial F ions. Various cluster structures have been envisaged containing 2–6 dopant ions, and their relative stabilities calculated by lattice simulation^{7,8}. One cluster involving a pair of dopants is shown in Fig. 1b. Stability is achieved by relaxations of the dopant and interstitial F ions in a $\langle 110 \rangle$ direction, towards the central cube edge, accompanied by a relaxation of the nearest pair of lattice anions outwards, in the $\langle 111 \rangle$ direction. The structure is catalogued as a $2|0|2|2_1$ cluster. This designation uses a systematic notation for clusters of the type $i|v|p|q_r$, in which i denotes the number of substitutional dopant ions, v the number of vacancies, p the number of relaxed lattice ions and q the number of interstitials in nearest-neighbour sites (for which $r=1$)⁸. Recent calculations predict that this cluster will readily trap a free interstitial fluoride ion, to form the relatively more stable, negatively charged $2|0|2|3_1$ cluster (Fig. 1c). The early neutron diffraction work of Cheetham *et al.*⁹ on Y-doped CaF₂ found evidence for the low-symmetry interstitial configuration predicted by the $2|0|2|2_1$ model. The more recent diffraction work of Catlow *et al.*⁵ supports the formation of the $2|0|2|3_1$ in La-doped CaF₂. However, several workers have suggested the formation of larger cubo-octahedral clusters^{10,11}. Figure 1d shows the hexameric $6|0|8|6_1 \langle 111 \rangle$ cluster, which the calculations show to

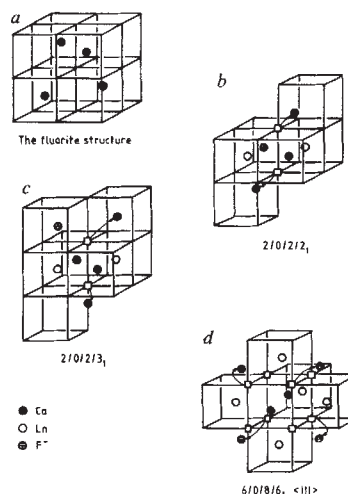


Fig. 1 *a*, The fluorite structure. *b*, The $2|0|2|2_1$ dopant dimer cluster. *c*, The $2|0|2|3_1$ dopant dimer cluster. The arrows show the postulated relaxation pathways of the lattice F ions. *d*, The $6|0|8|6_1 \langle 111 \rangle$ dopant hexamer. (Five of the six lanthanide dopants, and four of the F interstitials, sited opposite the edges of the central cube, are drawn in *d*. The arrows show how a 45° rotation of the central cube faces, followed by an outward relaxation, will generate 8 of the 14 F interstitials.

be the most stable of the clusters containing six dopant ions.

Detailed calculations of the relative energies of the different clusters discussed above revealed that the stability of the hexameric cluster, compared with that of the smaller dopant dimers and tetramers, increased markedly as the dopant ion radius decreased. Thus, at the high Z end of the lanthanide series, simulations predicted that the larger hexameric clusters will predominate. On the other hand, at the low Z end of the series, calculations suggested that no one particular cluster—dimer or hexamer—is preferred; the larger hexameric clusters should become the preferred structure at Gd in the series where the radius has dropped to 1.05 Å (ref. 8).

Some support for a change in cluster structure with dopant radius has been found in the neutron diffraction studies^{6,7} which show significant differences between interstitial structures in La-doped CaF₂ and Er-doped CaF₂. However, as noted, diffraction gives only average structural information; EXAFS, on the other hand, provides information on the local environment of the dopants and should give direct evidence for such a change in cluster structure. Furthermore, detailed structural models

Table 1 Radial distances in Ln/CaF₂ structures

No. of atoms	Type	RD from EXAFS (Å)	Debye-Waller factor σ^2 (Å ²)	Simulation RD (Å)
a				
10	F	2.40	0.014	2.40
1	Nd	3.97	0.013	3.79
11	Ca	3.91	0.024	3.87
1	F	4.18	0.005	4.05
24	F	4.50	0.028	4.53
6	Ca	5.45	0.026	5.46
24	F	5.95	0.030	5.95
24	Ca	6.69	0.035	6.69
b				
9	F	2.35	0.011	2.35
1	F	3.32	0.007	3.45
8	Ca	3.93	0.016	3.83
2	F	4.12	0.007	4.05
4	Er	4.21	0.023	4.05
22	F	4.54	0.034	4.55

Comparison of radial distances (RD) obtained from fitting: *a*, the Nd/CaF₂ data to the 2[0]2[3]₁ cluster configuration, and *b*, the Er/CaF₂ data to the 6[0]8[6]₁ cluster configuration, with the corresponding distances predicted by computer simulations.

from computer simulations are especially useful in interpreting the EXAFS of these disordered crystalline solids.

EXAFS spectra

Figure 2 shows the EXAFS spectra for the Ca_{0.9}Ln_{0.1}F_{2.1} series; Ln is an element in the rare-earth series La to Yb. In traversing this series, the frequency of the EXAFS oscillations decreases and the shape of the fine structure is modified: in particular, the shoulder on the peak around 50 eV shifts to lower energies, a shoulder appears on the high-energy side of the peak around 100 eV and the feature at 125 eV disappears. Qualitatively, the local structural environments of Gd–Yb in CaF₂ are essentially the same but differ from those displayed by La–Nd. Thus, the EXAFS data support the predictions of the computer simulations of a change in preferred structure about midway across the lanthanide series.

By taking the Fourier transform of the experimental data, it is possible to obtain a distribution function, similar to a radial distribution function, for the atoms surrounding the rare-earth ion^{12,13}. The difference between the two types of local structure surrounding the rare-earth defects can be seen in Fig. 3, where the Fourier transforms of Nd/CaF₂ and Er/CaF₂ are compared with that of Ca in CaF₂. All three distributions display three separate peaks in the range 0–5 Å. Whereas the structure surrounding Ca persists out to 10 and 11 Å, that surrounding the rare-earth dopants is less extensive. Nd displays some features beyond 5 Å at similar distances to those in CaF₂ but the atomic environment of Er is less well ordered. The histograms in Fig. 3 represent the radial distributions of atoms surrounding dopants for the most stable clusters predicted by the lattice simulation studies^{7,8} compared with that surrounding Ca in CaF₂. In all three environments, there are predominantly three shells in the range 0–5 Å which can be used to identify the peaks in the experimental EXAFS atomic distributions: 8 or 9 fluorines at 2.3–2.4 Å, 11 or 12 cations (Ca/Ln) at ~4 Å and a further 20–24 fluorines at ~4.5 Å. It is immediately obvious from Fig. 3 that the peaks for the second and subsequent shells of neighbours in the EXAFS distributions are much weaker for Nd and Er than for Ca. For shells with similar atomic weighting, there can be little doubt that the local fluorite lattice is considerably distorted by the presence of rare-earth dopants.

Electron phase shifts

The central problem in calculating the EXAFS of a postulated spectrum is to obtain accurate estimates of the phase shifts of the photoelectrons. These are functions of the photoelectron

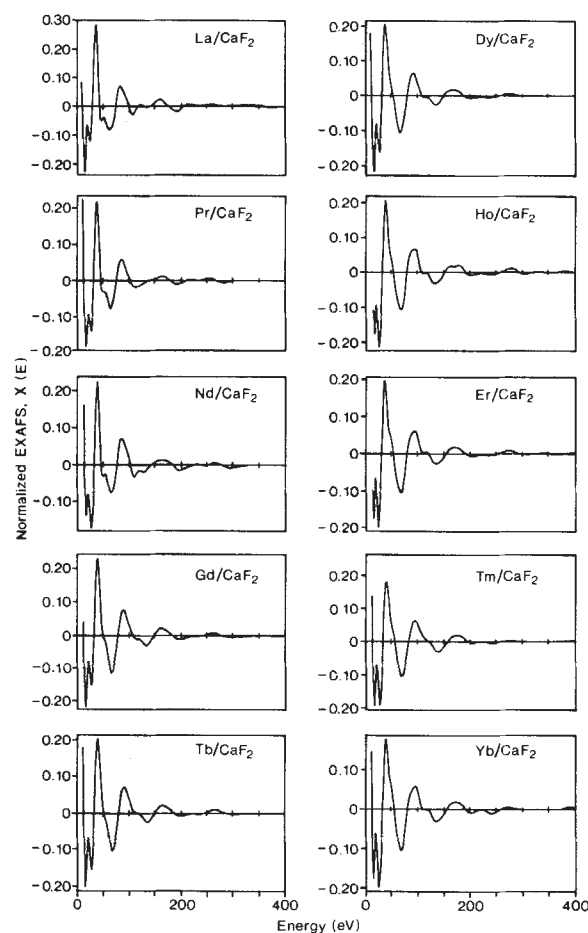


Fig. 2 The lanthanide, Ln(III), EXAFS for the series Ca_{0.9}Ln_{0.1}F_{2.1}. Single crystals of Ca_{0.9}Ln_{0.1}F_{2.1} were grown from 4N-purity powder LnF₃ (Rare Earth Products Ltd) and CaF₂ using a modified Stockbarger technique¹⁷. The rare earths used were Ln = La, Pr, Nd, Gd, Tb, Dy, Ho, Er, Tm and Yb. Portions of the single crystals were ground to a fine powder using a micronizing mill, then mixed with powdered polythene and pressed in a 13 mm die to form coherent pellets. The ratios of sample to polythene and pellet thicknesses were calculated so as to yield an absorption ratio, $\ln(I_0/I)$, of 1.20 at the energy of the rare-earth ion's Ln(III) absorption edge. The X-ray energies of the lanthanides used cover the range 5.49–8.95 keV. The EXAFS measurements were performed at room temperature in transmission mode, using the EXAFS station 7.1 on the SERC Synchrotron Radiation Source (SRS) at Daresbury. The SRS ran at 2 GeV with a typical beam current of 100 mA. An order-sorting two-crystal monochromator was used. Data analysis was carried out using the EXAFS program package on the Daresbury Laboratory NAS 7000 computer.

wave vector k (the momentum of the photoelectron ejected in the absorption process), and are related to the normalized EXAFS (χ) by the expression:

$$\chi(k) = \sum_j A_j(k) |f_j| \sin(2kR_j + \psi) \quad (1)$$

where each of the j shells of neighbours contributing to the EXAFS pattern has an amplitude term, A_j , depending predominantly on the number of atoms in that shell; R_j is the distance from the central atom to the atoms in the j th shell; and ψ is the total shift in phase imparted to the escaping photoelectron wave in passing through the atomic potentials of the central atom and the j th shell atoms. The amplitude function, $|f_j|$, describes the effectiveness of the atoms in the j th shell in backscattering photoelectrons of different momenta and can be calculated from the phase shifts^{14,15}. Thus, to determine the atomic positions accurately, it is necessary to obtain reliable phase-shift functions over the entire energy range of the EXAFS

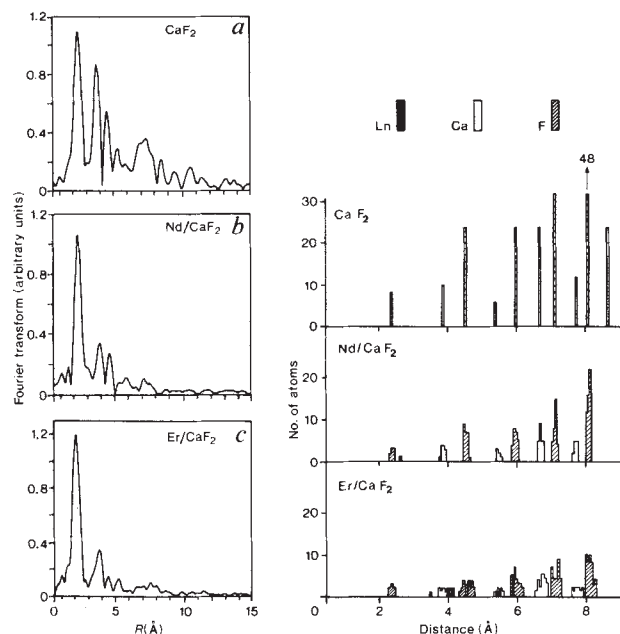


Fig. 3 Fourier transforms of the experimental data of: *a*, K edge of Ca in CaF_2 ; *b*, Ln(III) edge of Nd in $\text{Ca}_{0.9}\text{Nd}_{0.1}\text{F}_{2.1}$; *c*, Ln(III) edge of Er in $\text{Ca}_{0.9}\text{Er}_{0.1}\text{F}_{2.1}$. Also shown are histograms depicting the radial distribution function of the three cations for CaF_2 , and the computer simulations of the $2[0]2[3]_1$ cluster for Nd/CaF₂ and the $6[0]8[6]_{\langle 111 \rangle}$ cluster for Er/CaF₂.

spectrum. In the simplest case, the total phase shift for a particular atom pair (for example, Ca and F) can be obtained empirically using model compounds for which the local geometries are known¹⁶. Alternatively, multiple phase shifts can be calculated for individual atoms¹⁴. The main advantage of using calculated phase shifts is that the EXAFS of postulated structures can be calculated *ab initio* using the spherical-wave approximation, which is particularly important in analysing EXAFS spectra, such as for the fluorites measured here, where the energy range of discernible fine structure (0–400 eV) is limited. The principal disadvantage, however, is that the chemical components in the phase-shift functions are difficult to reproduce precisely. For example, phase shifts for Ca in a metallic environment will be slightly different from those in an ionic environment and the difference is difficult to calculate realistically from first principles.

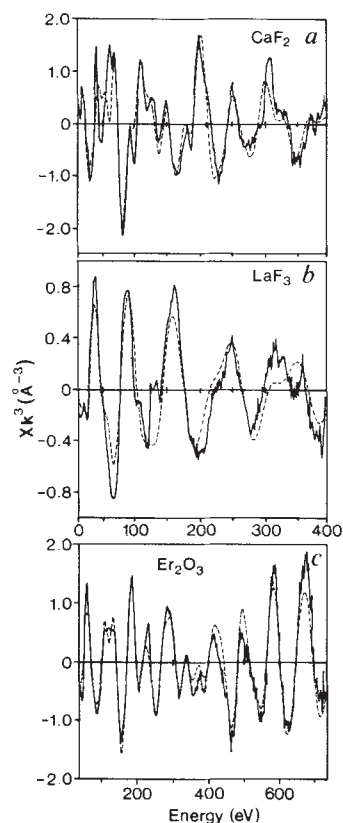
For these reasons, we have taken calculated phase shifts as starting parameters and have used least-squares methods to adjust their magnitude and k -dependence until the best fit to the experimental data for several model compounds is achieved (N. Binsted and L.M.M., in preparation). The structural standards chosen were CaF_2 , Er_2O_3 and LaF_3 in which the cations are in similar electronic and chemical environments as the samples. Figure 4 shows the experimental data (weighted by k^3 to enhance the oscillations at high energy) and the calculated EXAFS obtained using these phase shifts and the appropriate coordination numbers. The analysed shell radii for neighbouring atoms up to 5 Å from the central atom agree with crystallographic values to better than 0.03 Å. All three spectra display the characteristic complex beating indicative of the contribution to the EXAFS of many shells of neighbouring atoms, as can be seen in the Fourier transform of the CaF_2 data (Fig. 3a).

Lattice simulations

To test whether the observed transition in the lanthanide EXAFS series presented in Fig. 2 corresponds to a change from a dimer to a hexameric cluster, we have calculated the theoretical fine structure profile expected for the different structures.

Previous simulations^{6,7} predict that the distance from the rare-earth ion to its nearest fluoride neighbours is less in the

Fig. 4 Fits to structural standards for *a*, CaF_2 ; *b*, LaF_3 ; and *c*, Er_2O_3 . —, Experimental data with k^3 weighting; ---, theory. All fits give the correct radial distances to within 0.01 Å for neighbours out to 4.5 Å. Subsequent radial distances are within 0.15 Å of the correct distances. This discrepancy can be attributed to multiple scattering and core-hole relaxation effects.



hexameric clusters than in the smaller aggregates. This may be the driving force for the smaller lanthanides at the high atomic-weight end of the series to form these larger clusters. Another difference between the computed cluster structures lies in the second shell consisting of 12 Ca ions for pure CaF_2 ; for the dimeric clusters the second shell has 11 Ca ions and 1 rare-earth ion, whereas for the hexamers, 4 out of the 12 cations are dopant ions (Fig. 3).

Nd/CaF₂ and Er/CaF₂ exemplify the two types of EXAFS observed across the rare-earth series (Fig. 2). Figure 5 shows the k^3 -weighted spectra for each: note that the fine structure is less detailed than for CaF_2 (Fig. 4), which is symptomatic of the increase in disorder introduced into the lattice by the presence of the dopant rare-earth cations, as noted before when the Fourier transforms were compared.

In analysing the Nd/CaF₂ and Er/CaF₂ spectra, we observe a shortening of the first-shell radius. Figure 6 shows the inverse Fourier transform of the filtered first peak of each spectrum. The frequencies are clearly different. The Er/CaF₂ spectrum has the lower frequency and hence the shorter first-shell radius. The Ln–F distances determined using the corrected phase shifts are 2.40 Å and 2.35 Å for Nd/CaF₂ and Er/CaF₂, respectively. The first-shell radii predicted by lattice simulation calculations consist of 10 fluorines at 2.40 Å for the dimeric $2[0]2[3]_1$ cluster and 9 fluorines at 2.35 Å for the $6[0]8[6]_{\langle 111 \rangle}$ cluster.

The second important difference between the dimeric and hexameric clusters is in the second shell where there are one Ln and 11 Ca for dimers but for hexamers there are 4 Ln and 8 Ca. The amplitude of the backscattering factor for Ca is quite different from that of Ln so different occupancies of the cation shell around 3.9 Å will result in different fine structure. We find that this is indeed the case and is responsible for most of the differences between the measured spectra. Further evidence in support of larger rare-earth ions preferring smaller clusters is the enhanced contribution to the EXAFS from the third-shell F ions in the case of the Nd/CaF₂ spectra. The peak at ~4.5 Å in the Fourier transform is larger for Nd/CaF₂ than for Er/CaF₂; this is expected as larger clusters cause longer-range disordering with a concomitant increase in the variance of the value of the radial distance. This is reflected in an observed increase, for

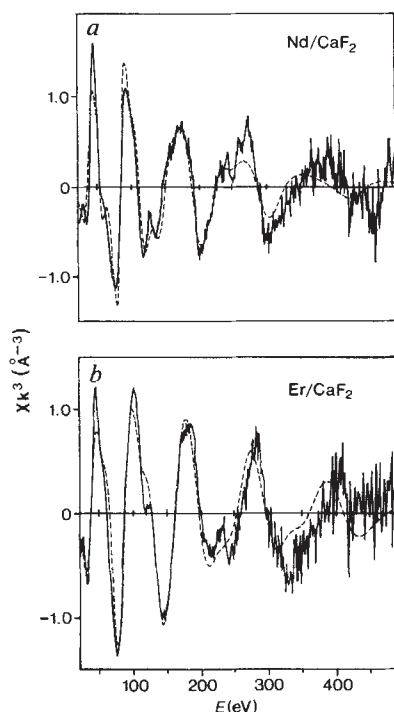


Fig. 5 The k^3 -weighted Ln(III) EXAFS of: *a*, $\text{Ca}_{0.9}\text{Nd}_{0.1}\text{F}_{2.1}$ and *b*, $\text{Ca}_{0.9}\text{Er}_{0.1}\text{F}_{2.1}$. The broken line shows the calculated EXAFS obtained using the parameters listed in Table 1.

Er/CaF_2 , of the Debye-Waller factor (see Table 1). This term contributes to A (equation 1) and accounts for loss of amplitude due to static as well as any thermally induced disorder.

The dashed curves in Fig. 5 demonstrate how the detailed differences between the dimer and hexamer cluster structures reproduce the observed differences in the EXAFS spectra of Nd and Er-doped CaF_2 . The theory reproduces the changes in structure of the peaks around 50 eV and 150 eV, including the

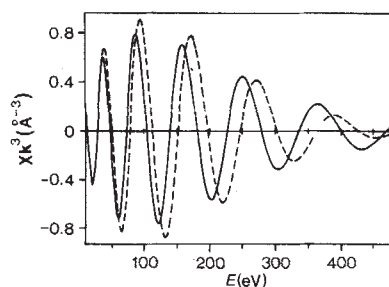


Fig. 6 A comparison of the Fourier-filtered first shell of F neighbours for Ln(III) EXAFS of $\text{Ca}_{0.9}\text{Nd}_{0.1}\text{F}_{2.1}$ (—) and $\text{Ca}_{0.9}\text{Er}_{0.1}\text{F}_{2.1}$ (---).

minimum around 140 eV. The fine structure between 200 and 300 eV is also accurately reproduced for the two structures. Details of the coordination number, shell radius and Debye-Waller factors are given in Table 1. Our results are in good agreement with the lattice simulation calculations. However, the accuracy in determining the radial distance to shells containing only one or two atoms is less than for shells having higher occupancies, owing to the smaller contribution of the former to the total spectrum. Lattice simulation calculations predict that the stabilization of the different clusters in the lighter lanthanides is very similar, leaving the possibility that a mixture of different clusters could be formed. Certainly, returning to Fig. 2, there are some small differences in the measured EXAFS amongst the La, Pr and Nd spectra, but the analysis of the Nd EXAFS spectrum (Fig. 5*a* and Table 1*a*) suggests that the presence of aggregations larger than the dimeric cluster is unlikely for the lighter lanthanides. Differences between the spectra for the heavier lanthanides are more subtle, in accordance with the greater stability of the hexameric cluster predicted by the calculations.

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Recombinant antibodies possessing novel effector functions

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The introduction into lymphocytes of immunoglobulin-gene DNA that has been manipulated in vitro allows the production of novel antibodies. In this way, cell lines have been established that secrete hapten-specific antibodies in which the Fc portion has been replaced either with an active enzyme moiety or with polypeptide displaying c-myc antigenic determinants.

MONOCLONAL antibodies secreted by hybrid myeloma cells¹ are used extensively both as diagnostic reagents² and in therapy³. Recent techniques allowing the stable introduction of immunoglobulin-gene DNA into myeloma cells⁴⁻⁶ make it possible to use *in vitro* mutagenesis and DNA transfection to produce

recombinant antibodies possessing novel properties. In particular, chimaeric proteins in which the antigen-binding portion of the immunoglobulin is fused to an enzymatic moiety could be of use in immunoassays. Furthermore, the chemical coupling of toxins to cell-specific antibodies has been advocated as a

Fig. 1 *a*, Structure of plasmids. Thin horizontal lines, pSV2gpt vector; thick lines, immunoglobulin-gene DNA; boxes, exons; hatched box, *S. aureus* nuclease coding region. The locations of the heavy-chain locus transcription enhancer element (E) and the *gpt* gene are indicated. Restriction endonuclease cleavage sites: R, *EcoRI*; Xh, *XhoI*; S, *SacI*; and Sl/Xh, a sequence formed by joining a *SalI* to an *XhoI* site. The sequence is presented around the *XhoI* site of plasmid pSV-V_{NP}γSNase, which forms the junction of the γ2b C_H2 exon and the SNase gene. *b*, Predicted structures of the anti-NP antibodies whose heavy chains are encoded by the plasmids in *a*. Disulphide linkages (—S—) between heavy (H) and light (λ) chain are indicated. Only one bivalent subunit of the decavalent pSV-V_{NP}-encoded IgM is illustrated.

Methods: In all three plasmids, the V_{NP} exons are contained on a common *EcoRI* fragment and the vector is the *BamHI*-*EcoRI* fragment of pSV2gpt (ref. 12) with an *XhoI* adapter in the *BamHI* site. pSV-V_{NP}γδ contains an *EcoRI*-*SacI* and pSV-V_{NP}γSNase an *EcoRI*-*XhoI* mouse Cγ2b fragment derived from phage λ MYG9 (ref. 13). Thus pSV-V_{NP}γδ contains the γ2b C_H1 and hinge exons whereas pSV-V_{NP}γSNase also contains the 5' end of the C_H2 exon. The mouse C_δ5 exon of pSV-V_{NP}γδ is contained in a *BamHI* fragment of phage Ch257.3 (ref. 14) which was obtained as a *SacI*-*SalI* fragment after subcloning in M13mp11. The SNase coding region derives from an M13mp8 clone containing a *Sau3A* fragment of *S. aureus* DNA inserted in the *BamHI* site; this clone was isolated from an M13 library by antibody screening (R.O.F., unpublished data). The DNA encoding the five amino acids linking the γ2b C_H2 domain and SNase derive from the SNase leader sequence. Removal of the SNase gene from the M13mp8 clone as a *BamHI*-*SalI* fragment and recloning in M13mp12W (ref. 15) allowed its isolation as a *XhoI*-*SalI* fragment for final assembly of pSV-V_{NP}γSNase.

technique in chemotherapy⁷; antibody fusions containing cytotoxic carboxy-terminal moieties could be exploited in this area.

Myeloma cells provide an attractive system for the production of chimaeric antibodies, as such cells are specialized in the synthesis and secretion of large amounts of immunoglobulin. However, at present, we do not know whether chimaeric antibodies can be secreted by a myeloma cell nor whether an antibody/enzyme fusion will retain both antigen-binding and enzymatic activity. We describe here experiments to test the feasibility of making recombinant antibodies in myeloma cells.

Reconstitution of hapten-specific antibody

We have described previously⁵ the construction of plasmid pSV-V_μl which includes a complete mouse immunoglobulin μ gene cloned in the expression vector pSV2gpt (Fig. 1). The immunoglobulin μ polypeptide encoded by this plasmid has a heavy-chain variable region, V_H, characteristic of λ1 light chain-bearing mouse antibodies that are specific for the hapten 4-hydroxy-3-nitrophenyl (NP); NP binding should therefore occur following association of the pSV-V_μl-encoded heavy chain with mouse λ1 light chains. To confirm this, pSV-V_μl DNA was introduced by spheroplast fusion into J558L, a mouse plasmacytoma expressing λ1 but producing no heavy chain⁴. Stably transfected cells were selected as described in Fig. 2 legend and were cloned by limiting dilution. Twenty clones were analysed by radioimmunoassay and each secreted high levels of an NP-specific IgM antibody (data not shown). Homogeneous anti-NP IgM antibody can be purified from supernatants of pSV-V_μl-transfected clones with a yield of about 3 mg l⁻¹ (Fig. 2).

Secretion of F(ab')₂-like antibody

To test whether deletion of the Fc portion of the heavy-chain gene results in impaired antibody synthesis or secretion, a derivative of pSV-V_μl was constructed in which the exons encoding

the μ-chain constant region (C_μ) were replaced by the C_H1 and hinge exons of the mouse γ2b gene. To provide translation termination and polyadenylation sequences, an exon, C_δ5, derived from the gene encoding secreted mouse δ chains, was placed at the 3' end of the gene (Fig. 1). The plasmid containing this truncated heavy-chain gene, pSV-V_{NP}γδ, would be expected to direct the synthesis of a F(ab')₂-like molecule consisting of two IgG2b Fab molecules disulphide-linked via the γ2b hinge and with a 21-amino acid tailpiece at the carboxy-terminus encoded by the C_δ5 exon.

Plasmid pSV-V_{NP}γδ was transfected into J558L cells and radioimmunoassay revealed that the stably-transfected cells secreted high levels of λ1-bearing anti-NP antibody. This NP-specific antibody was purified from culture supernatants of several transfected clones with a yield of 5–10 mg l⁻¹. Polyacrylamide gel electrophoresis of the purified material in non-reducing conditions (Fig. 2c) reveals a major protein species of relative molecular mass (M_r) ~110,000, which agrees well with the predicted M_r of a F(ab')₂-like molecule. Minor bands of M_r ~50,000 are also observed which may be from Fab' molecules. On reduction, a band co-migrating with λ light chain and several polypeptides of higher M_r are detected; the major band among these has an M_r of 31,000 and could constitute the heavy chain of the F(ab')₂-like antibody.

The minor antibody heavy-chain components do not reflect glycosylation heterogeneity, because the electrophoretic mobilities of the pSV-V_{NP}γδ-encoded heavy-chain bands are unaffected by inclusion of tunicamycin in the incubation medium during biosynthetic labelling experiments (Fig. 2b). As the protein samples analysed were purified on antigen columns, it is likely that the minor species differ from the F(ab')₂ antibody in the carboxy-terminal portion of the heavy chain, possibly as a result of alternative processing of pSV-V_{NP}γδ transcripts.

Nevertheless, it is clear that F(ab')₂-like anti-NP antibody can be synthesized and secreted in high yield by pSV-V_{NP}γδ-transfected J558L cells. This agrees with previous observations⁸ that

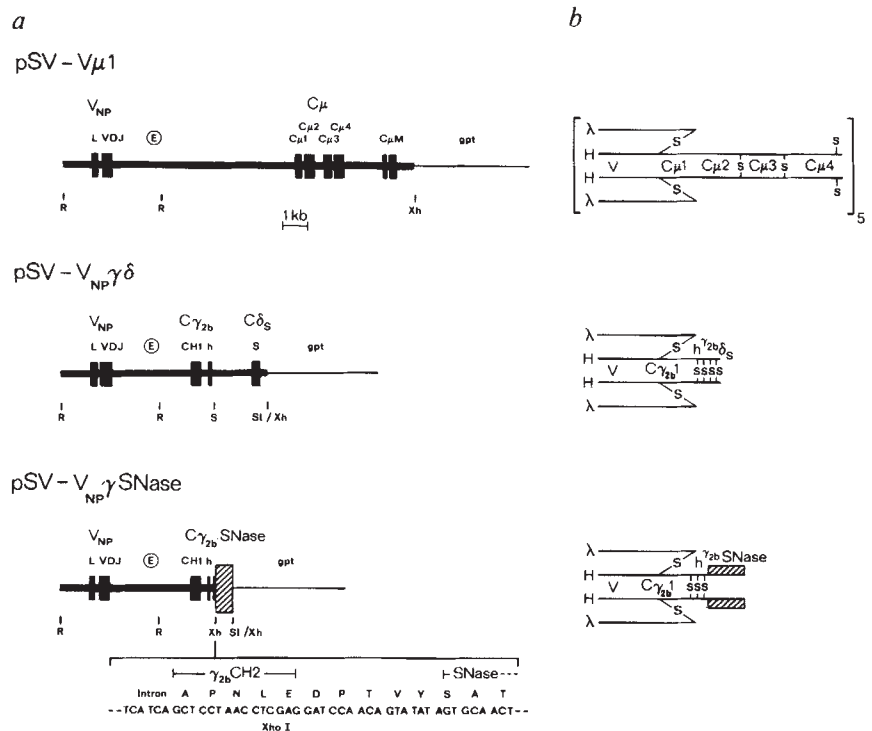


Fig. 2 SDS-polyacrylamide gel electrophoresis of anti-NP antibodies secreted by J558L transfectants. *a*, Purified antibody (30 μ g) boiled with 2-mercaptoethanol before electrophoresis and stained with Coomassie blue; *b*, biosynthetically labelled antibody analysed after reduction. Samples purified from cells labelled in the presence of tunicamycin are indicated (Tm); *c*, unreduced antibody (30 μ g) purified from culture supernatants.

Methods: J558L cells stably transfected with the pSV plasmids were obtained by spheroplast fusion and selected essentially as described previously⁵ except that hypoxanthine-aminopterin-thymidine was omitted from the selective medium and mycophenolic acid was used at 5 μ g ml⁻¹, as described by Oi *et al.*⁴. Transfection frequencies of 10⁻⁴ to 10⁻³ were obtained: in all cases, cells were cloned by limiting dilution. Antibody samples were purified from supernatants of J558L transfectants grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Supernatants (2 l) were passed over 2-ml columns of 4-hydroxy-5-iodo-3-nitrophenacetyl-aminocaproic Sepharose (NIPcap-Sepharose) and antibody was eluted from the washed sorbent with 1 mM NIPcapOH in phosphate-buffered saline. Biosynthetically-labelled antibody was purified on 40 μ l NIPcap-Sepharose columns from supernatants of cells incubated for 4 h at 37 °C in medium containing L-³⁵S-methionine. Tunicamycin (8 μ g ml⁻¹) was, if required, included during a 2-h preincubation as well as during the labelling itself; parallel incubations with an IgE-secreting cell line confirmed the efficacy of the drug. Reduced samples were analysed on 12% polyacrylamide gels and 7% gels were used for unreduced samples.

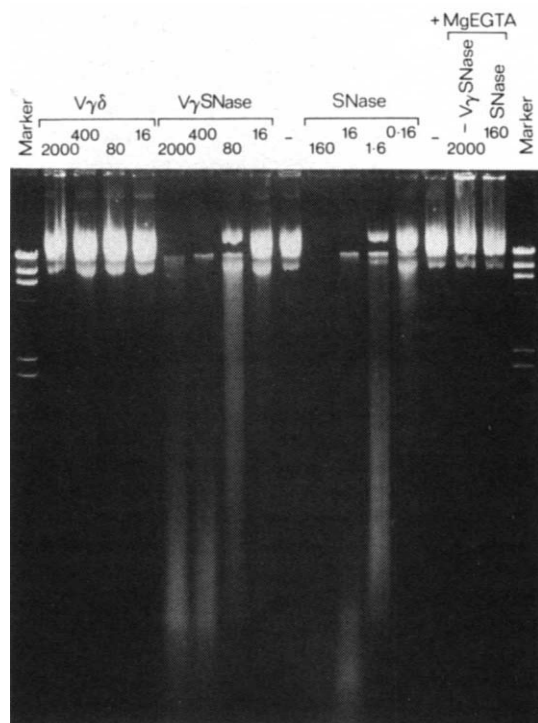
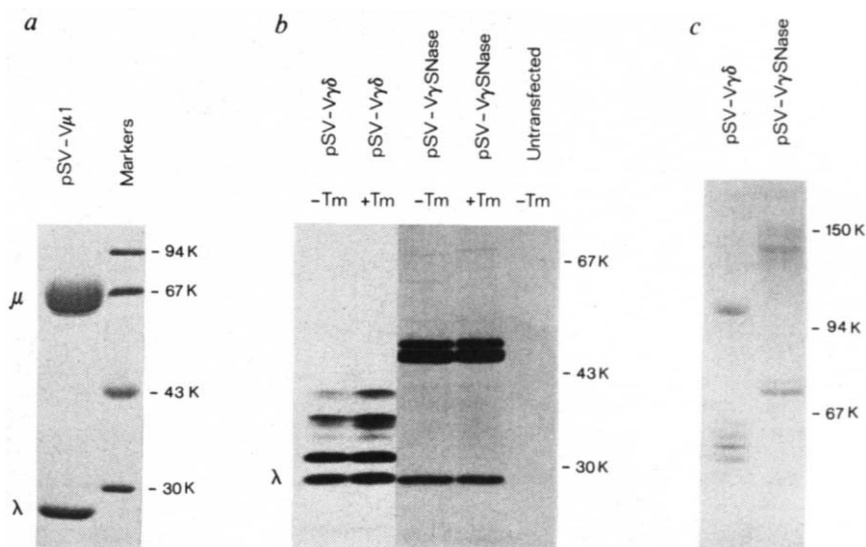


Fig. 3 Assay for nuclease activity. Single-stranded M13 DNA (2 μ g) was incubated at 37 °C for 30 min in 25 mM sodium borate, 250 mM NaCl, 10 mM CaCl₂, pH 8.5 (20 μ l) containing various amounts of V_{NP} γ δ or V_{NP} γ SNase antibody or of purified *S. aureus* nuclease. The quantities of antibody/enzyme used are in ng. DNA in the samples was then analysed by ethidium bromide fluorescence after electrophoresis through a 1.2% agarose gel. A HindIII digest of phage λ DNA provides size markers. Ca²⁺-dependency of the nuclease activity was confirmed by running incubations in the presence of 40 mM MgCl₂ and 25 mM EGTA.

monovalent recombinant Fab-like molecules can be secreted by transfected myeloma cells.

Secretion of Fab-nuclease

As a novel activity to fuse to the antibody heavy chain, we chose the nuclease from *Staphylococcus aureus*, selected because it is a secreted enzyme active as a monomer, contains no thiol

residues and can easily re-fold after denaturation⁹. A DNA restriction fragment containing the *S. aureus* nuclease (SNase) gene was inserted into the *Xho*I site located in the C_H2 exon of the mouse γ 2b gene. Plasmid pSV-V_{NP} γ SNase was assembled as described in Fig. 1 legend; the heavy-chain gene of pSV-V_{NP} γ SNase is similar to that of pSV-V_{NP} γ δ except that the C δ _s exon has been removed and replaced by an exon containing the first five codons of the γ 2b C_H2 exon fused in phase to the nuclease-coding region. Simian virus 40-derived sequences of the pSV2gpt-derived vector provide polyadenylation signals. J558L cells were transfected with pSV-V_{NP} γ SNase and cells surviving in selective medium were cloned by limiting dilution.

Radioimmunoassay of supernatants of cloned transfectants revealed that about one-third were positive for the production of λ -bearing anti-NP antibody. Positive clones yielded 1–10 mg l⁻¹ of NP-binding antibody. Analysis of biosynthetically-labelled antibody by gel electrophoresis reveals a band comigrating with λ 1 light chain as well as two heavy-chain bands of *M*_r 45,000 and 46,000 (Fig. 2b). The difference between these two heavy chains has not been identified but their mobilities agree well with the predicted mobility of the V_{NP} γ -SNase heavy chain. Although the sequence Asn-Asn-Thr is present in SNase, the two V_{NP} γ SNase heavy-chain bands are still present in samples purified from supernatants of cells that have been biosynthetically labelled in the presence of tunicamycin (Fig. 2). This demonstrates that the difference between them is not due to N-linked glycosylation. The V_{NP} γ SNase antibody seems somewhat more heterogeneous on a non-reduced gel, giving bands with the expected mobilities of both the F(ab')₂-SNase and Fab-SNase. The presence of SNase on the heavy-chain carboxy-terminus might inhibit disulphide linking of the γ 2b hinge regions.

Enzymatically active Fab-nuclease

To test for nuclease activity in the V_{NP} γ SNase preparation, samples purified on hapten-Sepharose columns were incubated with single-stranded DNA substrate. Digestion of the DNA was monitored following agarose gel electrophoresis. V_{NP} γ SNase but not V_{NP} γ δ antibodies show nuclease activity and this activity, like that of authentic *S. aureus* nuclease, is dependent on Ca²⁺ but not Mg²⁺ ions (Fig. 3). As judged on a molar basis, the catalytic activity of the V_{NP} γ SNase sample is ~10% that of authentic *S. aureus* nuclease.

The V_{NP} γ SNase antibody can be used as a genetically conjugated enzyme-linked antibody in enzyme-linked immunosorbent

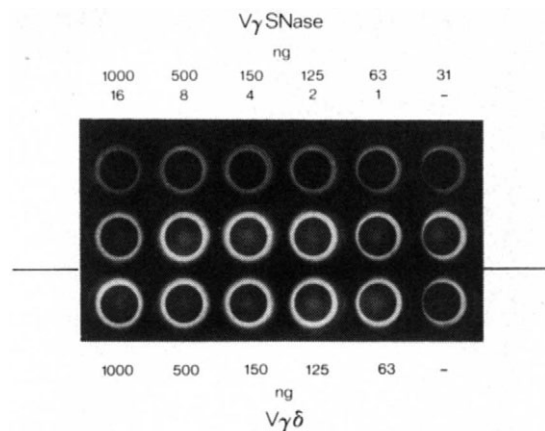


Fig. 4 Use of $V_{NP}\gamma$ SNase antibody in ELISA-type assay. Polyvinyl microtitre plates were coated with $40 \mu\text{g ml}^{-1}$ (NIP)₂₀-bovine serum albumin (BSA). After blocking unreacted sites with BSA, dilutions of $V_{NP}\gamma$ SNase or $V_{NP}\gamma\delta$ antibodies were incubated in the wells; ng of antibody incubated in each well are indicated. After washing off unbound material, a solution ($40 \mu\text{l}$) containing $1 \mu\text{g}$ M13 single-strand DNA and $1 \mu\text{g ml}^{-1}$ ethidium bromide in pH 8.5 buffer (see Fig. 3 legend) was added. The plate was photographed after 1 h incubation at 37°C .

assays (ELISAs). Antigen-coated plastic plates were incubated with various amounts of $V_{NP}\gamma$ SNase protein and bound antibody was then detected by its nuclease activity, by adding to the plate a solution containing DNA and ethidium bromide. Following digestion of the DNA substrate by the immobilized $V_{NP}\gamma$ SNase antibody, the fluorescence due to the DNA/ethidium bromide complex substantially decreased. Concentrations of $\sim 10 \text{ ng}$ $V_{NP}\gamma$ SNase antibody are detected easily; no decrease in fluorescence is obtained with the $V_{NP}\gamma\delta$ antibody control (Fig. 4). The assay may be made at least 10-fold more sensitive by increasing the incubation time with the DNA substrate.

Secretion of Fab-myc

As discussed above, the nuclease is an attractive moiety to fuse to an antibody because of its stability, ease of renaturation and

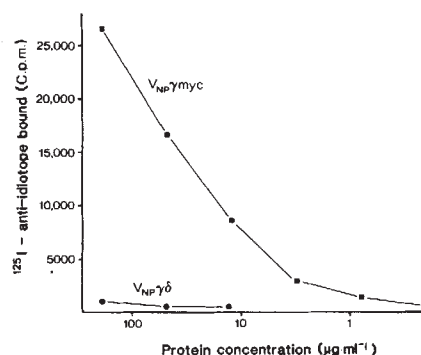


Fig. 6 Assay for *c-myc* antigenic determinant in Fab-*myc*. Monoclonal anti-*c-myc* antibody CT14-G4.3 (gift of G. Evan) was coated ($10 \mu\text{g ml}^{-1}$) onto polyvinyl microtitre plates and unoccupied sites were then blocked with BSA. Varying concentrations of either anti-NP Fab-*myc* (■) or $F(ab')_2$ (●) were incubated in the wells and, after washing, bound anti-NP antibody was detected by developing with radioiodinated monoclonal anti-idiotypic antibody Ac38. The Ac38 antibody (gift of M. Reth and K. Rajewsky) recognizes the variable region of the anti-NP antibodies¹⁶. Controls performed with Ac38-reactive monoclonal IgM, IgD and IgD antibodies ($200 \mu\text{g ml}^{-1}$) gave less than 400 c.p.m.

lack of thiol groups and the fact that it is itself a secreted protein. We therefore decided to fuse the carboxy-terminal portion of the mouse oncogene *c-myc* to the antibody Fab to test the generality of the approach. The product of the *c-myc* gene is a protein which contains many thiol groups and normally exists in the cell. There is no reason to believe that the third exon of *c-myc* on its own will encode a functional protein domain. Thus, if the Fab-*myc* fusion protein were secreted by the cell, not only would it provide a source of protein for making anti-*myc* antisera, but also it would allow us to assess the general feasibility of secreting chimaeric antibodies.

Plasmid pSV- $V_{NP}\gamma$ myc was assembled as described in Fig. 5 legend; the plasmid is similar in structure to pSV- $V_{NP}\gamma\delta$ except that the $C_{\delta 8}$ exon is replaced with the 3'-terminal exon of the mouse *c-myc* gene. This *c-myc* exon encodes 187 amino acids¹⁰ and should provide the transcription polyadenylation signal. The plasmid was transfected into J558L and cells from wells positive for production of anti-NP antibody were cloned by limiting dilution. Hapten-binding protein was purified from culture supernatants and analysed for the presence of *c-myc* antigenic determinants in an indirect radioimmunoassay. Samples of either the putative Fab-*myc* or the anti-NP $F(ab')_2$ (to act as control) were incubated in wells of a polyvinyl microtitre plate that had been coated with a monoclonal anti-*c-myc* antibody; bound anti-NP antibody was then detected using a radioiodinated monoclonal anti-idiotypic antibody which recognizes the Fab portion of the anti-NP antibodies.

The Fab-*myc* clearly binds to the monoclonal anti-*c-myc* antibody, whereas the anti-NP $F(ab')_2$ and other controls do not (Fig. 6). The Fab-*myc* was also recognized by two other monoclonal antibodies specific for the carboxy-terminal end of *c-myc* (data not shown). SDS-polyacrylamide gel electrophoresis (Fig. 5) of the Fab-*myc* reveals that it is somewhat heterogeneous; a band co-migrating with $\lambda 1$ light chains and several bands with a higher M_r of 38,000–55,000 are observed, without a single dominant heavy-chain band being apparent. The expected size of the Fab-*myc* is M_r 50,000. We have observed that, after prolonged storage, precipitates appear in the Fab-*myc* sample and SDS-polyacrylamide gel electrophoresis reveals more extensive heterogeneity of the heavy-chain bands. We therefore believe that the heterogeneity of the Fab-*myc* protein indicated by the SDS-polyacrylamide gel analysis may be caused by proteolytic degradation.

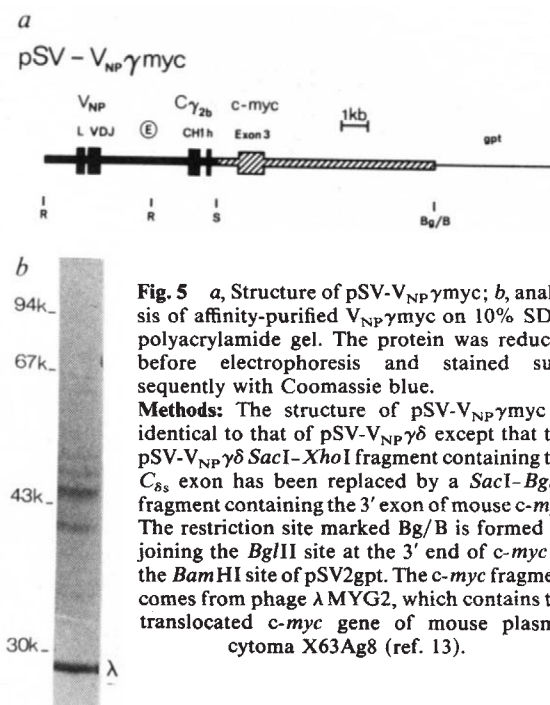


Fig. 5 *a*, Structure of pSV- $V_{NP}\gamma$ myc; *b*, analysis of affinity-purified $V_{NP}\gamma$ myc on 10% SDS-polyacrylamide gel. The protein was reduced before electrophoresis and stained subsequently with Coomassie blue.

Methods: The structure of pSV- $V_{NP}\gamma$ myc is identical to that of pSV- $V_{NP}\gamma\delta$ except that the pSV- $V_{NP}\gamma\delta$ *SacI*-*XhoI* fragment containing the $C_{\delta 8}$ exon has been replaced by a *SacI*-*BglII* fragment containing the 3' exon of mouse *c-myc*. The restriction site marked Bg/B is formed by joining the *BglII* site at the 3' end of *c-myc* to the *BamHI* site of pSV2gpt. The *c-myc* fragment comes from phage λ MYG2, which contains the translocated *c-myc* gene of mouse plasmacytoma X63Ag8 (ref. 13).

Conclusion

Myeloma cells can synthesize and secrete active recombinant antibodies. The Fab-nuclease is secreted in good yield, retains enzymatic activity and is a stable protein. We have shown how such an antibody/enzyme fusion protein can be used in immunoassay and it is clear that analogous recombinant antibodies could find application in other fields. The results obtained with the Fab-*myc* construct suggest that although it may be possible to drive secretion of a large variety of different substitutions of the Fc portion, problems may be encountered with the stability of the resultant protein; in this case it is probable that the *c-myc*-encoded part of the antibody is unable to fold as a protein domain. Nevertheless, the fact that *c-myc* antigenic

determinants are clearly displayed by the Fab-*myc* protein suggests that this might be a valid approach to the preparation of antisera against the previously unidentified products of specific genes.

Finally, it is worth noting that these antibody/enzyme fusions may provide an attractive means for driving the synthesis and extracellular secretion of specific enzymes as fusion proteins which could then be purified easily on antigen columns. The enzyme and antibody moieties could, if required, be dissociated using specific proteases¹¹.

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Adenovirus-2 E1A products repress enhancer-induced stimulation of transcription

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The adenovirus-2 early region 1A (E1A) products repress activation of transcription induced by the simian virus 40, polyoma virus and adenovirus-2 E1A enhancers. The repression probably involves an interaction between the enhancer elements and a trans-acting factor(s), possibly the E1A products themselves.

ENHANCERS are promoter elements, unique to eukaryotes, which potentiate transcription from other homologous or heterologous 'natural' or 'substitute' promoter elements (such as the TATA box), either in the presence or absence of upstream (distal) promoter elements (refs 1-5 and references therein). Viral and cellular enhancers exhibit species and/or cell specificity^{1,2,4,6-8}, suggesting that their activity may be positively regulated by factors acting in *trans*. Recent studies from our laboratory have shown that stimulation of transcription *in vitro* by the simian virus 40 (SV40) enhancer involves the formation of a stable complex between a *trans*-acting factor and the enhancer element⁹⁻¹¹. No example of the negative regulation of an enhancer element has yet been described. However, we found previously¹² that when HeLa cells were co-transfected with a recombinant containing the rabbit β -globin gene linked to the polyoma virus (Py) enhancer and a recombinant expressing the adenovirus-2 (Ad-2) early region 1A (E1A) gene products, there was a marked decrease in the amount of RNA initiated from the β -globin gene promoter. This suggested that the E1A products might block the activity of the Py enhancer. Such a possibility is intriguing, as it is known that the E1A products encoded in 13S and 12S messenger RNAs are involved in the activation of transcription from all other early viral transcription units¹³⁻¹⁵, as well as in cell immortalization and cell transformation¹⁶⁻¹⁹. Moreover, transcription from cellular promoters can be stimulated by the E1A products when recombinants containing these promoters are either co-transfected with the E1A transcription unit into HeLa cells or transfected into 293 cells that express

constitutively an integrated E1A gene (refs 20-22 and references therein).

We demonstrate here that the E1A products repress the activity of the SV40, Py and Ad-2 E1A enhancers, and that this inhibition probably involves an interaction between the enhancer elements and a *trans*-acting factor(s), possibly the E1A products themselves.

E1A products repress enhancer activity

We used the recombinant plasmid pE1ASV (Fig. 1; ref. 12), containing the entire E1A transcription unit including the enhancer sequences²³⁻²⁵, to study the effect of the E1A products on the transcription of two enhancer-containing recombinants. The first of these, p β (244+) β , contains the Py enhancer linked to two rabbit β -globin genes; the second, pSVBA34 (ref. 5), contains the SV40 enhancer (72 base pair (bp) repeat region) linked to a fragment of the Ad-2 major late promoter (Ad-2MLP) and the SV40 large-T antigen coding region (Fig. 1). When increasing amounts of pE1ASV were co-transfected into HeLa cells with a constant amount (10 μ g) of the p β (244+) β recombinant, a progressive decrease in RNA transcribed from the β -globin genes was observed (Fig. 2a, Glob+1; b, lanes 1-3). A sixfold decrease was obtained with 100 ng pE1ASV (Fig. 2a, lane 4), corresponding to a ratio of 1 molecule of pE1ASV plasmid to 30 molecules of the polyoma β -globin recombinant. In parallel experiments, the stimulation of transcription from the E2a transcription unit by the E1A products was assayed by co-transfecting pE1ASV and pE2G (a chimaeric recombinant containing

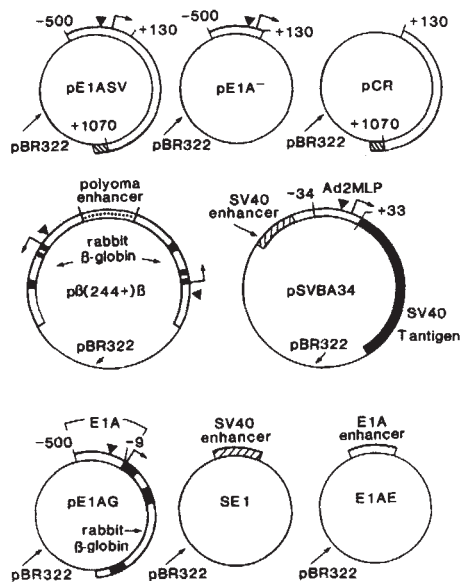


Fig. 1 Structure of the recombinants. pE1ASV (4.6 kilobases (kb), see ref. 12) contains the 1,570 left-most nucleotides of Ad-2. The E1A transcription unit from positions -500 to +1,070 with respect to the E1A cap site is linked to the 135-bp SV40 *Hpa*I (2,604)/*Bam*HI (2,469) fragment containing the SV40 polyadenylation signal (hatched box). pE1A⁻ (3.4 kb, see ref. 12) and pCR (4 kb) were derived from pE1ASV and contain respectively the E1A promoter region (positions -500 to +130) or the coding region (positions +130 to +1,070). pE1AG (5.2 kb, a gift of C. Goding and C. Kédinger) contains the E1A promoter from positions -500 to -9 linked by a *Hind*III linker to the rabbit β -globin gene (from positions -9 to +1,650 with respect to the β -globin cap site). p β (244+) β (13.1 kb, see ref. 37) contains the polyoma viral enhancer (fragment *Pvu*II (5,262)-*Bcl*I (5,021)) and two copies of the rabbit 4.5 kb chromosomal fragment including the rabbit β -globin gene³⁸. pSVBA34 (7.7 kb, see ref. 5) contains the SV40 enhancer (coordinates 113-270) linked to a fragment of the Ad-2 major late promoter (Ad-2MLP) (-34 to +33 with respect to the major late cap site) and to the SV40 large-T antigen coding region (coordinates 2,533-5,227). SE1 (3 kb) (see ref. 11) contains the SV40 enhancer (coordinates 113-270) cloned between the *Pvu*II and *Bam*HI sites of pBR322. E1AE (4.5 kb) contains the E1A enhancer sequences from position -500 to -145 (with respect to the E1A cap site) cloned between the *Eco*RI and the *Bam*HI sites of pBR322 (see ref. 11). None of the recombinants is drawn to scale. Solid boxes in pE1AG and p β (244+) β indicate β -globin exons.

the E2a promoter and the rabbit β -globin coding sequence; see ref. 15). The results show clearly that the decrease in RNA transcribed from p β (244+) β and the activation of the E2a promoter occur in the same range of pE1ASV concentration (Fig. 2d).

The low molar ratio at which inhibition occurs suggests that it is not due to competition between the promoters of the two co-transfected recombinants. This hypothesis is supported by the results of co-transfection experiments with recombinant pE1A⁻, containing the E1A promoter region but not the E1A coding sequences (Figs 1 and 2b, lanes 4, 5), or recombinant pE1AG, in which the E1A coding region is replaced by the β -globin coding region (Figs 1 and 2c, lane 3). In neither case was there a decrease in the amount of RNA transcribed from the β -globin mRNA start site of p β (244+) β . In addition, *in vitro* nuclear run-on transcription experiments have shown previously that pE1A⁻ is more efficiently transcribed than pE1ASV (see ref. 15). Thus, competition between the promoters of the co-transfected recombinants cannot account for the decrease in transcription from the β -globin promoters of p β (244+) β . To exclude the possibility that the E1A coding region might contain a sequence trapping factors required for Py enhancer activity,

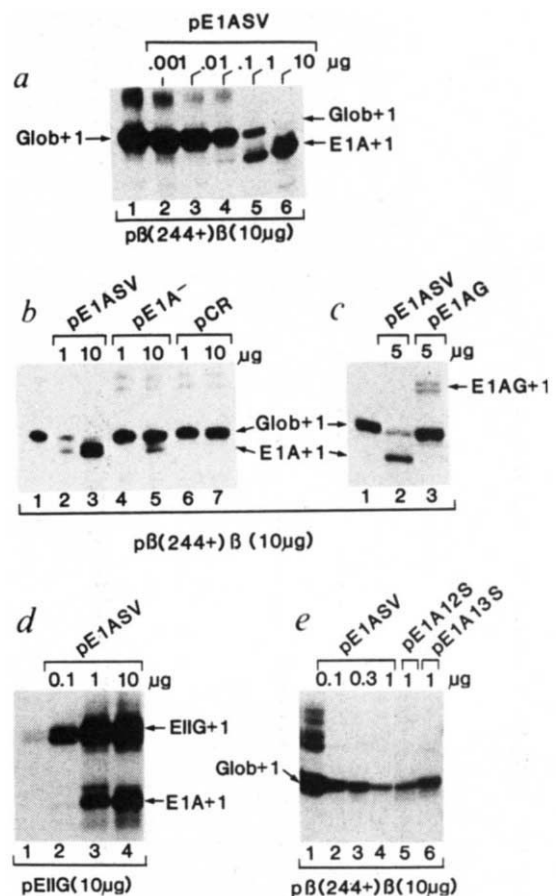


Fig. 2 Quantitative S_1 nuclease analysis of cytoplasmic RNA extracted from HeLa cells co-transfected with 10 μ g recombinant p β (244+) β and pE1ASV, pE1A⁻, pCR, pE1AG, pE1A12S or pE1A13S (a, b, c, e); d, 10 μ g recombinant pE1IG (see text and ref. 15) was co-transfected with increasing amounts of pE1ASV. The recombinants co-transfected and the amount of DNA transfected are indicated above the gel lanes. Transfection was carried out using the calcium phosphate procedure⁵ with the following modification: the cell monolayer was washed after 24 h and the cells collected 16 h later. The total amount of transfected DNA was kept constant at 20 μ g by the addition of pBR322. Cytoplasmic RNA was prepared⁵ and 10 μ g RNA hybridized with excess 5'-end labelled E1A and globin single-stranded probes^{5,12}, except in c, lane 3, and e, where the globin probe alone was used. The E1A probe is an *Eco*RI(-500)/*Sau*3A(+130) fragment and the β -globin probe a *Bst*NI fragment as described previously³². After S_1 nuclease treatment, the E1A-protected fragment (E1A+1), corresponding to RNA transcribed from pE1ASV or pE1A⁻, was 130 nucleotides in length. The globin-protected fragment (Glob+1), corresponding to RNA transcribed from p β (244+) β , was 134 nucleotides long, whereas RNA transcribed from pE1AG (E1AG+1) or pE1IG (E1IG+1) protected a 140-nucleotide long fragment. The decrease in RNA transcribed from the β -globin genes of p β (244+) β was estimated by densitometric scanning of autoradiograms corresponding to several experiments similar to a. Average decreases of 6-, 40- and 200-fold were obtained with 100 ng, 1 μ g and 10 μ g of co-transfected pE1ASV, respectively.

we constructed recombinant pCR, containing only the E1A coding sequences (Fig. 1). No decrease in β -globin RNA is observed when pCR is co-transfected with p β (244+) β (Fig. 2b, lanes 6, 7).

We next co-transfected pE1ASV with pSVBA34, a recombinant in which transcription from the +33 to -34 Ad-2MLP element is stimulated by the SV40 enhancer (Fig. 1). We found a decrease in RNA initiated from the Ad-2MLP, similar to those obtained previously with p β (244+) β (Fig. 4a, b, lanes 1, 2, data not shown). To examine the individual roles of the 12S and 13S mRNA products of the E1A transcription unit, we

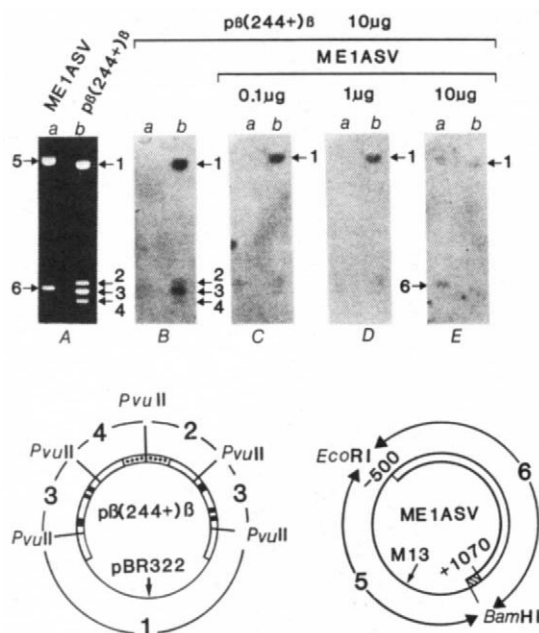


Fig. 3 Nuclear run-on transcription from nuclei of cells transfected with recombinants p $\beta(244+)\beta$ and ME1ASV. Lower part, ME1ASV was derived from pE1ASV by replacing the pBR322 sequences with M ϕ 11 sequences using the polylinker single *Eco*RI and *Bam*HI sites. Upper part, restriction digests (2 μ g DNA) of p $\beta(244+)\beta$ (lanes b) and ME1ASV (lanes a) were electrophoresed on a 1.5% agarose gel and blotted onto nitrocellulose filters as indicated. A, Ethidium bromide-stained agarose gel. Fragments 1–6 (see lower part) are 6.5, 1.8, 1.65, 1.5, 7.2, 1.7 kb in length, respectively. Fragment 3 contains the two β -globin genes of p $\beta(244+)\beta$. B–E, Autoradiograms of the nitrocellulose blots after hybridization with labelled nuclear run-on RNAs prepared from HeLa cells transfected with p $\beta(244+)\beta$ alone (B) or with ME1ASV (as indicated).

Methods: p $\beta(244+)\beta$ was transfected into HeLa cells with increasing amounts of ME1ASV; nuclei were isolated, incubated *in vitro* (in the presence of heparin and ammonium sulphate) with 32 P-CTP and cold GTP, UTP and ATP and, after purification, RNA was hybridized to the above DNA fragments, as described in ref. 15.

co-transfected Py or SV40 enhancer-containing recombinants with cDNA-derived E1A recombinants producing either the 13S or the 12S mRNA (see ref. 15). Both E1A, 12S and 13S mRNA products can inhibit Py and SV40 enhancer activity (Fig. 2e, lanes 5, 6, and data not shown).

To rule out the possibility that the E1A products do not inhibit transcription activation by the enhancers, but rather interfere with the stability or the transport of the RNA produced, we performed run-on transcription experiments on isolated nuclei (Fig. 3). Co-transfection of 10 μ g p $\beta(244+)\beta$ with as little as 0.1 μ g of ME1ASV (a recombinant in which the pBR322 sequence of pE1ASV was replaced by M13 sequences) results in inhibition of transcription of the β -globin genes (Fig. 3B–E). As, in these nuclear run-on transcription experiments, the RNA synthesized corresponds only to elongation of RNA chains previously initiated *in vivo* (see ref. 15), we conclude that the E1A products repress the enhancer-mediated activation of β -globin gene transcription. In fact, a decrease in transcription from the other sequences of the recombinant p $\beta(244+)\beta$ is also observed (Fig. 3, fragments 1, 2, 4). The residual level of transcription obtained with p $\beta(244+)\beta$ when repression is maximal (10 μ g ME1ASV, Fig. 3E), corresponds to the level obtained with a recombinant lacking the enhancer sequence (data not shown). This suggests strongly that the E1A products repress all enhancer-activated transcription in recombinant p $\beta(244+)\beta$, including transcription initiated not only from the β -globin promoters, but also from pBR322 'substitute' promoter elements³.

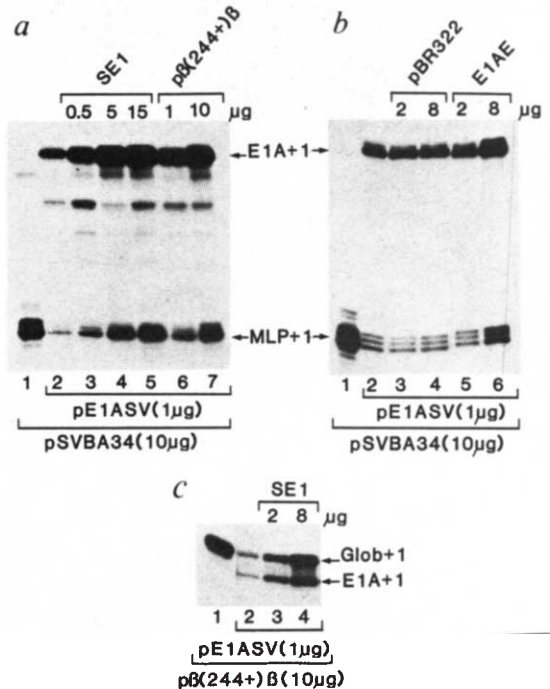


Fig. 4 Co-transfection with recombinants containing the Py virus or SV40 enhancers relieves repression of enhancer-mediated transcription by the E1A products. The experimental procedure is described in Fig. 2 legend. a, b, Lanes 1, 10 μ g pSVBA34 was transfected alone. a, b, Remaining lanes, pSVBA34 and pE1ASV were transfected in the amounts indicated. In a, lanes 3–7, b, lanes 3–6, a third recombinant was co-transfected. The nature of this recombinant and the amount of DNA transfected are indicated above the gel lanes. c, 10 μ g p $\beta(244+)\beta$ was transfected either alone (lane 1) or with 1 μ g of pE1ASV (lanes 2–4). A third recombinant, SE1, was co-transfected in lanes 3 and 4 as indicated. RNA transcribed from pE1ASV (E1A+1) and p $\beta(244+)\beta$ was analysed by quantitative S₁ nuclease mapping as indicated in Fig. 2 legend. The probe used for analysing RNA initiated from pSVBA34 at the Ad-2MLP cap site was the 5' end labelled single-stranded *Hind*III-*Xho*I fragment (see ref. 5) giving a protected fragment of 93 nucleotides (MLP+1).

Involvement of *trans*-acting factor(s)

To investigate whether repression of the enhancer-mediated stimulation of transcription by the E1A products is a *trans*-acting process involving an interaction with the enhancer, we attempted to remove the repression by co-transfecting increasing amounts of a third recombinant containing only a purified enhancer element cloned in pBR322. Increasing amounts of SE1 (a recombinant in which the SV40 enhancer is cloned in pBR322, see Fig. 1) in co-transfections where transcription of pSVBA34 (Fig. 4a, lanes 2–5) or p $\beta(244+)\beta$ (Fig. 4c, lanes 2–4) is repressed by pE1ASV, derepresses transcription initiated either from the Ad-2MLP (MLP+1 in Fig. 4a, compare lanes 1–5) or from the β -globin promoter (Glob+1 in Fig. 4c, compare lanes 1–4). The molar ratios of SE1 : pSVBA34 are 0.13, 1.3 and 3.9 in lanes 3–5 of Fig. 4a, whereas those of SE1 to p $\beta(244+)\beta$ are 0.9 and 3.6 in lanes 3 and 4 of Fig. 4c. Similarly, the addition of the Py enhancer in the form of the recombinant p $\beta(244+)\beta$ to a transfection also containing pSVBA34 or pE1ASV relieves the repression of transcription from the Ad-2MLP (Fig. 4a, compare lanes 1, 2, 6 and 7; the molar ratios of p $\beta(244+)\beta$ to pSVBA34 are 0.17 and 1.7 in lanes 6 and 7, respectively). In contrast, no 'derepression' is observed when pBR322 is transfected with pSVBA34 and pE1ASV (Fig. 4b, lanes 3, 4). We conclude from these results that repression of enhancer activity by the E1A products may involve an interaction between the enhancer and either the E1A products or some cellular factor(s) induced by the E1A products, or a combination of both.

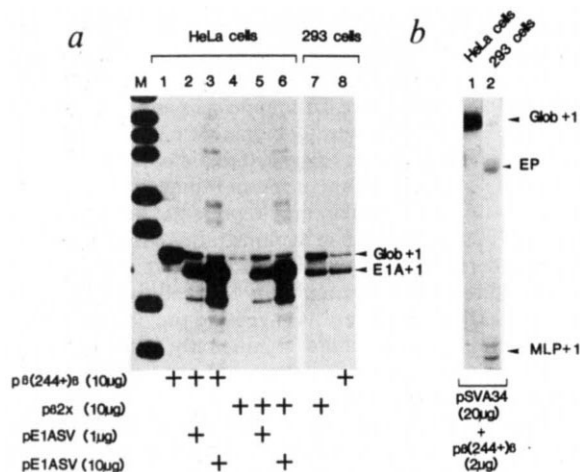


Fig. 5 Quantitative S_1 nuclease analysis of RNA synthesized in HeLa or 293 cells transfected with $p\beta(244+)\beta$, pSVA34, p $\beta 2X$ and pE1ASV as indicated. pSVA34 (ref. 5) is similar to pSVBA34 (Fig. 1), except that it does not contain the SV40 enhancer. p $\beta 2X$ is similar to $p\beta(244+)\beta$ (Fig. 1) except that it does not contain the polyoma virus enhancer (see ref. 37). The probes used for S_1 nuclease mapping of RNA initiated from the Ad-2MLP (MLP+1), the β -globin promoter (Glob+1) or the E1A promoter (E1A+1) are described in Figs 2 and 4 legends. Hybridization reactions contained either 10 μ g cytoplasmic RNA from HeLa cells or 20 μ g cytoplasmic RNA from 293 cells. Other experimental procedures are described in Fig. 2 legend. EP, end point of homology between transcripts initiating in pBR322 and the Ad-2 major late probe.

In the derepression experiments (Fig. 4a, c), the amount of RNA initiated at the E1A mRNA start site (E1A+1) increases in parallel with that of RNA initiated from either the Ad-2MLP (Fig. 4a, lanes 3–7) or the β -globin promoter (Fig. 4c, lanes 3, 4). These results suggest that E1A products can also repress transcription stimulated by the E1A enhancer(s)^{23–25}. This hypothesis is supported by the increase of RNA initiated from the Ad-2MLP (MLP+1) and E1A promoter (E1A+1) when a recombinant containing the E1A enhancer region inserted in pBR322 (E1AE, see Fig. 1) is transfected with pSVBA34 and pE1ASV (Fig. 4b, compare lanes 1, 2, 5 and 6).

E1A stimulation of enhancer-less promoters

E1A products expressed either permanently in the 293 cells transformed by the adenovirus-5 E1 genes, or transiently in HeLa cells after transfection with the Ad-2 E1A transcription unit, can stimulate transcription from a variety of genes transfected into these cells. Strikingly, transcription can be stimulated in these conditions not only from the other adenovirus early genes^{15,22,25}, but also from recombinants containing cellular transcription units^{20–22,26}. The E1A products thus appear to act as activators of transcription from RNA polymerase class B (II) promoters, perhaps by interacting with the TATA box region or the factor(s) recognizing it²⁰. Interestingly, the SV40 enhancer has no effect on rabbit β -globin gene expression in 293 cells²¹ or in HeLa cells²² co-transfected with a recombinant expressing the E1A products. In the latter case, the presence of SV40 enhancer even results in a two- to threefold decrease in RNA transcribed from the rabbit β -globin gene.

There is no real discrepancy between our results and those published previously (Fig. 5a; refs 20–22). E1A products clearly stimulate transcription from the rabbit β -globin gene promoter in the absence of an enhancer (Fig. 5; compare lanes 4–6, where p $\beta 2X$, a derivative of $p\beta(244+)\beta$ that does not contain an enhancer, is transfected either alone or with increasing amounts of pE1ASV). In identical transfection conditions, co-transfection of the Py enhancer-containing rabbit β -globin recombinant $p\beta(244+)\beta$ and the plasmid expressing the E1A products (pE1ASV) strikingly decreases transcription from the β -globin

promoter (Fig. 5, lanes 1–3). At the higher pE1ASV concentration, the amounts of β -globin RNA synthesized from the enhancer-containing $p\beta(244+)\beta$ recombinant (Fig. 5, lane 3) and the enhancer-less p $\beta 2X$ recombinant (Fig. 5, lane 6) are very similar. This suggests that when a recombinant expressing the E1A products (pE1ASV) is co-transfected with an enhancer-containing β -globin gene recombinant $p\beta(244+)\beta$, the E1A products abolish the activity of the enhancer, but are still capable of stimulating transcription from the β -globin promoter. These observations may explain also previous conclusions that an enhancer does not increase the amount of RNA transcribed from the rabbit β -globin gene in HeLa cells co-transfected with a plasmid expressing the E1A products²².

It is also clear that in 293 cells, the rabbit β -globin gene is not expressed at a higher level in a recombinant containing the Py enhancer (Fig. 5a, lane 8) than in a recombinant lacking it (p $\beta 2X$; Fig. 5a, lane 7). (The amount of β -globin RNA in lane 8 should be corrected for a lower content in E1A RNA when compared with the amount of β -globin RNA signal in lane 7.) The stimulatory effect of the Py enhancer is cancelled in 293 cells, whereas the activity of the β -globin promoter of the enhancer-less recombinant p $\beta 2X$ is still stimulated by the E1A products synthesized in these cells (Fig. 4; compare lanes 1 and 4 with lanes 7 and 8). However, as there are no control cells for 293 cells, these results cannot be directly compared with those obtained with pE1ASV-transfected HeLa cells. Similar results are obtained when the Py enhancer-containing recombinant $p\beta(244+)\beta$ and the Ad-2MLP enhancer-less recombinant pSVA34 (pSVBA34 lacking the SV40 enhancer; Fig. 1) are co-transfected into HeLa or 293 cells (Fig. 5b). The level of expression of these two recombinants in HeLa and 293 cells cannot be compared directly because a co-transfected reference plasmid cannot be used. However, in agreement with the above conclusion, there is a clear inversion in the relative level of expression of these recombinants in HeLa and 293 cells. Relative to the enhancer-containing recombinant $p\beta(244+)\beta$, the enhancer-less pSVA34 plasmid is expressed more efficiently in 293 cells (Fig. 5b, lane 2), whereas, relative to pSVA34, $p\beta(244+)\beta$ is better transcribed in HeLa cells (Fig. 5b, lane 1).

Conclusion

The results described here demonstrate that the stimulation of transcription mediated by enhancers can be negatively regulated. Because it is possible to compete the negative effect of the E1A products by co-transfecting recombinants containing only an enhancer element, and also because repression of the enhancer activity does not interfere with the ability of the E1A products to stimulate transcription from the β -globin promoter, the enhancer itself is probably the target for the negative regulation. Future studies will determine whether the E1A products themselves, or a cellular product(s) induced by them, or some combination of the two, interact with the enhancer sequence and/or with other enhancer factors. Using an extensive set of SV40 enhancer mutants (M. Zenke, T. Grundström, H. Matthes, M. Wintzerith and P.C., manuscript in preparation), we will determine which enhancer sequences are involved in E1A repression. In this respect, the only sequence feature which is apparently shared by the SV40, Py virus and Ad-2 enhancers is the core sequence^{23,27}. *In vivo* studies have clearly shown that the SV40 enhancer can generate an altered chromatin structure over its own sequence²⁸. Recent *in vitro* studies demonstrate that a transcriptional factor(s) interacts specifically with the SV40 enhancer^{9–11}. We are now investigating whether the E1A products interfere with either of these enhancer functions.

At present it is not known whether the E1A products inhibit the activity of the E1A enhancer during lytic infection or whether the inhibition could be observed only with an isolated E1A transcription unit. However, inhibition of the E1A enhancer activity by the E1A products provides a means by which the expression of the E1A transcription unit can be autoregulated during lytic infection. Indeed, it has been reported that E1A transcriptional activity is highest during the early phase of

infection^{13,14}. On the other hand, there is evidence that the E1A products stimulate transcription of the E1A transcription unit early in adenovirus infection^{29,30}. It appears, therefore, that the E1A products can act as both negative and positive regulators of their own transcription units during lytic infection, and as positive regulators of all other viral transcription units (see refs 13–15). Note that the SV40 early gene product, the large-T antigen, also regulates its own synthesis, but by a more 'classical' repression mechanism that does not involve an interaction with the SV40 enhancer³¹. In addition, like the E1A products, the large-T antigen is a pleiotropic protein also activating transcription, as it has been reported recently that it stimulates the activity of the SV40 late promoter³². It is not known whether inhibition of cellular enhancers by the E1A products occurs during productive infection or during cell transformation by adenoviruses. As yet, the expression of only one defined gene has been shown clearly to be repressed by E1A products. The expression of a class I major histocompatibility complex (MHC) gene is repressed in rat cells transformed by the E1A transcription unit of the highly oncogenic adenovirus-12 (Ad-12), but not by that of the weakly oncogenic adenovirus-5 (ref. 19). Since the E1A products of Ad-12 also inhibit activation of transcription by SV40 and Py enhancers (our unpublished observations), we are now investigating the possibility that this class I MHC gene contains an enhancer whose activity can be repressed by the Ad-12 E1A products.

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A bacterial repressor protein or a yeast transcriptional terminator can block upstream activation of a yeast gene

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A bacterial repressor protein blocks transcription of a gene in yeast when its operator is placed in the promoter between the upstream activator sequence and the transcription start point. Putative transcription terminators from yeast installed in the same region of the promoter have a similar effect.

PROMOTERS recognized by RNA polymerase II of the yeast *Saccharomyces cerevisiae* contain two essential elements: a stretch of DNA containing a sequence homologous to TATA, and an upstream activator sequence (UAS)¹. For example, synthesis of *GAL1* mRNA, which begins 80 nucleotides downstream of the TATA region of the *GAL1* promoter, depends on a sequence called UAS_G located about 250 nucleotides upstream

of the TATA region²⁴ (see Fig. 1). UAS_G function has been localized to a 75-nucleotide sequence², and, more recently, to a 15-base pair (bp) sequence with approximate 2-fold rotational symmetry (E. Giniger *et al.*, in preparation). The *GAL4* gene product is thought to bind to this sequence in the presence of galactose to activate transcription downstream. When it is placed in front of the TATA region of *CYC1*, UAS_G activates transcrip-

Gene expression in eukaryotic cells may be controlled by regulatory factors repressing enhancer activity. This has not yet been reported. However, the Py enhancer is not active in undifferentiated embryonal carcinoma cells, although mutations in the region containing the Py enhancer restore its activity in these cells^{33–35}. It has been suggested that embryonal carcinoma cells express a protein with properties similar to those of the E1A products³⁶. This protein may repress the activity of the Py enhancer. Thus, in addition to demonstrating that the adenovirus E1A products have both negative and positive regulatory functions, our study raises the possibility that cellular enhancers are regulated not only positively as suggested previously^{1,2,4,6–8}, but also negatively, which would significantly increase the combinatorial possibilities for the control of gene expression at the transcriptional level in eukaryotes.

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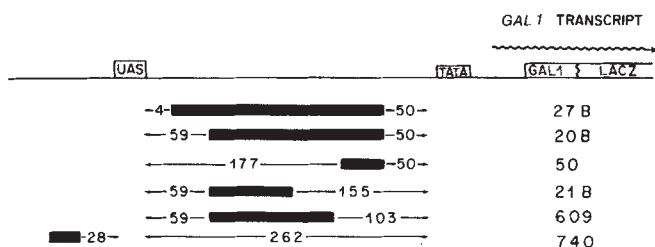


Fig. 1 Derivatives of the *GAL1* promoter. All carried a *XhoI* site into which *lexA* operators were inserted. Distance from the *XhoI* site to the TATA region and to the upstream activator sequence for various promoter derivatives is given in nucleotides. The *UAS_G* region contains two homologous 15-nucleotide regions of approximate 2-fold rotational symmetry separated by three nucleotides (one of which is sufficient for *UAS_G* function; E. Giniger *et al.*, in preparation). When defined in this way, there are 262 nucleotides between *UAS_G* and the first base of the TATA sequence. Numbering in *GAL1* derivatives is from the centre of the *XhoI* recognition sequence to the closest base in the upstream activator sequence, and to the first T of the *GAL1* TATA sequence. The mature *GAL1* transcript begins 85 nucleotides downstream of this T⁴, and the *GAL1* coding sequence begins 60 nucleotides farther downstream^{2,4}. The extent of promoter deleted in each derivative is shown by a dark bar. 36 nucleotides are deleted in 740. Plasmids containing sites for *lexA* operator insertion were made by combining members of two sets of deletions². 609 and 740 were constructed as described in ref. 2. 20B and 27B are described in ref. 2. 50 and 21B were gifts of M. S. Lamphier.

tion from the hybrid promoter and renders this transcription inducible by galactose⁵. The distance between *UAS_G* and a TATA region is not critical, and the effect of *UAS_G* is still measurable even when it is located 500 nucleotides upstream of a TATA region (R. Yocum, personal communication). In these respects, *UAS_G* shares properties with the transcriptional enhancer sequences found in higher eukaryotes.

We describe two different ways to block *GAL1* promoter function by interposing two different kinds of genetic elements between its upstream activator sequence and its TATA region.

We constructed a strain of yeast that synthesizes *lexA* protein, a repressor protein from *Escherichia coli*. We also constructed variants of the yeast *GAL1* promoter that carried *lexA* operators at different positions within the promoter. We found that those *GAL1* promoters carrying a *lexA* operator between the upstream activator sequence and the TATA region were repressed by *lexA* protein.

We next installed sequences located downstream of the coding regions of the *CYC1* and *ADH1* genes between the *GAL1* upstream activator sequence and its TATA region. These sequences diminished *GAL1* transcription. A fragment of DNA containing a mutation interfering with proper *CYC1* termination and differing from the terminator fragment by a single base pair, was less able to decrease *GAL1* transcription.

LexA protein represses transcription

LexA protein is a repressor of many of the genes induced in *E. coli* as a consequence of DNA damage⁶⁻⁹. A plasmid directing the synthesis of LexA protein in yeast was constructed (Fig. 2). Use of anti-LexA antiserum allowed us to detect LexA protein in extracts of yeast carrying this plasmid. We estimate that the LexA protein comprises 0.005–0.025% of the total protein when yeast containing this plasmid are grown on galactose-containing medium. We inserted synthetic *lexA* operators into derivatives of the *GAL1* promoter (described in Fig. 1 legend) and chose a *lexA* operator sequence based on analysis of the sequence of many LexA protein-binding sites and on some of their interactions with the protein. Gel analysis of DNA–protein complexes¹⁰ (K. Chapman and R.B., unpublished data) revealed that the synthetic 22-nucleotide operator bound LexA protein at least as tightly as any single naturally occurring operator thus far

Table 1 LexA protein repression of *GAL1* promoter with *lexA* operators

	Control plasmid	<i>lexA</i> plasmid
20B	5,000	5,000
+1 op	2,500	350
+2 op	2,500	250
27B	1,800	1,800
+1 op	1,000	150
50	5,000	5,000
+1 op	2,500	600
+2 op	2,400	500
21B	5,000	5,000
+1 op	2,500	400
+2 op	2,300	250
609	5,000	5,000
+1 op	3,100	400
740	5,000	5,000
+1 op	5,000	5,000

Yeast was transformed with two types of plasmids having two different selectable markers. One plasmid directed the synthesis of LexA protein and carried the *LEU2* marker. Another plasmid contained a derivative of the yeast *GAL1* promoter with a *lexA* operator site and carried the *URA3* marker. Transformed yeast were isolated and propagated on medium that selected for cells bearing both plasmids. The amount of transcription from the *GAL1* promoter was measured after cultures of doubly transformed cells had been grown on galactose-containing medium for at least five generations. The derivatives of the *GAL1* promoter in which *lexA* operators were installed are shown in Fig. 1. Synthetic *lexA* operators with the sequence

TCGAGTACTGTATGTACATACAGTAC

CATGACATACATGTATGTCATGAGCT

were inserted directly into *XhoI*-cut *GAL1* promoter derivatives (shown in Fig. 1) using standard plasmid construction techniques¹⁹. To construct a given doubly transformed strain, DNA from one of these plasmids and DNA from either pAAH5 (control plasmid) or pRB500 (which directed the synthesis of LexA protein) was used to transform strain DBY 745 to *URA⁺* and to *LEU⁺*²⁰. The amount of *GAL1* transcription in a given strain was estimated from indicator plates²⁸. The numbers in this table and in the tables below refer to units of β -galactosidase measured in liquid assays as described in ref. 3. Each strain was assayed in triplicate on at least five different occasions. Normally the level of β -galactosidase in a given transformed strain varied by no more than 20% on the day of the assay. Assay-to-assay variation of the ratio of galactosidase activity from any two different strains was usually less than 10%. Measured units were averaged and rounded to the nearest 50 units. op, Operator.

Table 2 Effect of LexA protein on transcription of an operator containing *GAL1* promoter integrated into the chromosome

Control plasmid	2,000
<i>lexA</i> plasmid	200

Plasmid 118 (20B+2 *lexA* operators) was partially digested with *EcoRI*, ligated, and the ligation mixture cut with *XbaI* to linearize plasmids that retained the *EcoRI* fragment from 118 containing the 2 μ origin. The ligation mixture was used to transform *E. coli* strain JM101²¹, which lacks a functional *lacZ* gene. 427, a derivative of 118 that lacks the *EcoRI* fragment containing the replication origin, was isolated from a *lacZ⁺*, Amp^R colony. 427 was cut in its *URA3* gene with *ApaI* and lithium acetate-treated DBY745 was transformed (see Table 1 legend) with the cut plasmid DNA. Six *URA* transformants arising from this construction were checked to see that the *URA* marker was not lost after growth for 40 generations on nonselective medium, and that *GAL1* promoter transcription in these strains was normally inducible if the yeast were grown on medium containing galactose. These six strains were made competent with lithium acetate, then transformed to *LEU⁺* with a plasmid directing the synthesis of LexA protein, pRB500, or with its parent plasmid, pAAH5. β -Galactosidase in these strains was assayed, in liquid and on indicator plates, as described in Table 1. There was no variation in *GAL1* transcription among the six strains when grown on galactose, glucose, and galactose with *GAL1* transcription repressed by LexA protein.

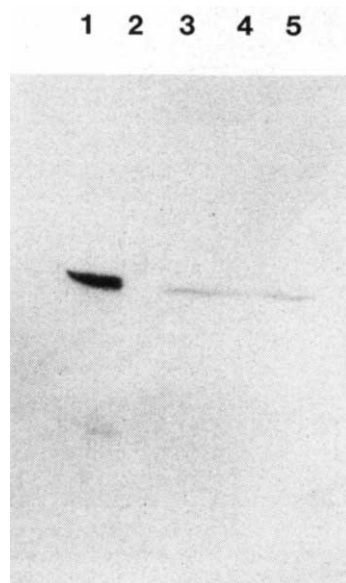


Fig. 2 *E. coli* LexA protein manufactured in yeast. Lane 1, 1 μ g of purified LexA protein⁹. About 10% of this LexA protein preparation consisted of the amino- and carboxy-terminal autolytic fragments²³. The carboxy-terminal fragment is visible on the gel. Lane 2, extract from DBY745/pAAH5. Lane 3, extract of DBY 745/pRB500. Lane 4, extract of DBY 745/pRB500. Lane 5, extract of DBY 745/pAAH5, spiked with 50 ng purified LexA protein.

Methods: pRB500, a plasmid which directs the synthesis of LexA protein, was made by inserting a 1,000-nucleotide *Hind*III-*Hind*III piece from pRB480 (unpublished data), which contained DNA from 62 bases upstream to ~200 nucleotides downstream of *lexA*, into the *Hind*III site of pAAH5. pAAH5 is a plasmid which carries the *LEU2* gene, a 2 μ origin of replication, and several hundred nucleotides of DNA from around the *ADH1* (*ADCI*) promoter. The *ADH1* fragment ends 11 nucleotides upstream of the ATG that would begin the *ADH1* structural gene. At this site a *Hind*III linker has been inserted. pAAH5 was constructed by Gustav Ammerer. Yeast strain DBY745 (α *adel-100 leu2-3 leu2-112 ura3-52*) was transformed with pAAH5 or pRB500, using the lithium acetate technique (M. Rose, personal communication). To produce each extract, yeast were grown in glucose minimal medium without leucine¹⁸ to a density of 2×10^7 ml⁻¹. 2 ml of this culture were concentrated by centrifugation, resuspended in 0.2 ml Laemmli sample buffer²⁴, and lysed by grinding with glass beads²⁵. 0.05 ml of each extract was run on a 12% polyacrylamide/SDS gel²⁴. Proteins in the gel were transferred electrophoretically to a nitrocellulose membrane; the membrane was probed with anti-LexA protein antibody followed by ¹²⁵I-protein A from *Staphylococcus aureus*¹³. The membrane was washed and exposed to XAR-5 film for 24 h. In other experiments, protein gels of lysates of yeast that carry this plasmid were stained with Coomassie brilliant blue and revealed a faint new band whose mobility was the same as that of pure LexA protein (not shown). LexA protein was also observed in these strains after cellular proteins were labelled with ³⁵S-methionine and immunoprecipitated with anti-LexA antiserum (not shown). From immunoprecipitation experiments and from stained gels, we estimated that LexA protein constituted between 0.02% and 0.1% of the total soluble protein in cells transformed with this plasmid grown in glucose. As the *ADH1* promoter is about five times less active when cells are grown in galactose as when cells are grown in glucose (refs 26, 27 and our unpublished results), we suspect that there is one-fifth this amount of LexA protein in galactose-grown yeast.

examined^{7,9}, with an apparent dissociation constant (K_d) of $<10^{-9}$ M. The *GAL1* promoter derivatives were carried on plasmids that supplied a replicator and a selectable marker. The *GAL1* structural gene was fused in each case to a *lacZ* gene so that the β -galactosidase level in a plasmid-carrying cell was a measure of the *GAL1* transcription.

Derivatives of the *GAL1* promoter containing *lexA* operators between the upstream activator sequence and the TATA region (derivatives 27B, 20B, 50, 21B and 609; Fig. 1) were repressed

Table 3 Putative transcriptional terminators, installed between the *GAL1* upstream activator sequence and its TATA region, decrease *GAL1* transcription

20B	5,000
+ <i>cycl-512</i>	2,500
+ <i>cycl-512-F</i>	800
+ <i>ADH1</i> terminator	50
+ 82-nucleotide polylinker	5,000
+ 234-nucleotide pBR322 <i>Hae</i> III fragment	5,000

Plasmids carrying *cycl-512* and *cycl-512-F* *Rsa*I. pAAH5 was cut with *Hind*III and *Sph*I. Fragments containing putative transcriptional terminators, the 182-nucleotide *Eco*RV-*Rsa* fragment, and the 324-nucleotide *Hind*III-*Sph*I fragment, were isolated and inserted into the *Sst*I site of pRB486 (unpublished construction). The unique *Sst*I site in this plasmid arises from the juxtaposition of two *Xho*I linkers. Derivatives of pRB486 that carried each mutation were cut with *Xho*I and the *Xho*I-ended terminator fragments inserted into the *Xho* site of 20B. Compared with the wild-type promoter, the *GAL1* promoter derivative carried on 20B has 153 nucleotides missing. Derivatives of 20B that carried these terminator fragments in the wild-type orientation were used to transform DBY745. A similar strategy was used to insert into 20B an 82-nucleotide *Hind*III-*Hae*III polylinker fragment from plasmid 701 (unpublished), a derivative of puc19²¹. 20B-G is a derivative of 20B that contains, in its *Xho*I site, a 234-nucleotide *Hae*III fragment from pBR322 (ref. 22). This plasmid was constructed by Jun Ma. Yeast bearing these plasmids were grown on galactose and assayed on indicator plates and in liquid as described in Table 1 legend.

4–10-fold by LexA protein (Table 1). All *GAL1* promoter derivatives containing two *lexA* operators were repressed more efficiently than corresponding derivatives that contained only a single operator. No repression was observed when the operator was installed upstream of the *UAS_G* (in *GAL1*, derivative 740; see Fig. 1). In the absence of LexA protein, *lexA* operators at certain sites in the *GAL1* promoter slightly diminished transcription (Table 1). This effect may be related to the slight homology between the *lexA* operator and sequences implicated in yeast transcription termination (unpublished data).

We sought to confirm that the apparent *lexA*-mediated repression of operator-containing *GAL1* promoters was not caused by variation in plasmid copy number. Accordingly, we constructed nonreplicating derivatives of two *lexA*-repressible *GAL1* plasmids and directed their integration into a yeast chromosome²⁹. We examined six different integrants of each plasmid and found that repression by LexA protein was as efficient, but no more efficient, than the repression observed when the operator-containing *GAL1* promoter was present in many copies on a plasmid. Repression of one such integrated promoter is shown in Table 2.

Upstream terminators decrease transcription

Sequences downstream of certain yeast genes have been identified tentatively as sites where transcription terminates; it is possible, although less likely, that these sequences are signals which cause a longer transcript to be processed¹¹⁻¹². Putative transcription terminators are found downstream of the coding sequences of the *CYC1* and *ADH1* (*ADCI*) genes (see Fig. 3 legend). We inserted fragments of DNA carrying these putative terminators or mutant derivatives of them between the *GAL1* upstream activator sequence and its TATA region. We placed the terminator fragments in a deletion derivative of the *GAL1* promoter to ensure that the distance between the upstream activator sequence and the TATA region in the terminator-containing construction was approximately that found in the wild-type promoter. One fragment was taken from the 3' end of a *CYC1* gene (the allele *cycl-512-F*) in which the correct messenger RNA (mRNA) is formed. Another fragment differs by a single base pair and was taken from a mutant (*cycl-512*) in which the correct 3' end of the mRNA is only formed inefficiently, presumably because termination is rendered defective by the changed base pair (refs 11, 12 and K. Zaret, M. Hampsey and F. Sherman, personal communication). In addi-

tional experiments, we used a fragment that carried the putative transcriptional terminator from the 3' end of the *ADH1* gene¹².

The fragments derived from the two *CYC1* alleles differed in their ability to depress *GAL1* transcription. The fragment carrying the putative transcriptional terminator (*cyc1-512-F*) decreased *GAL1* transcription by a factor of six (Table 3). In contrast, the fragment derived from the termination-deficient mutant (*cyc1-512*) decreased transcription by a factor of two. The fragment derived from *ADH1* showed a larger effect. Installation of this piece of DNA decreased *GAL1* transcription by 100-fold. Control pieces of DNA from pBR322 and pUC19, installed in the same deletion, had no effect on *GAL1* transcription (Table 3). If the *CYC1* terminator is inserted between the *CYC1* upstream activator sequence and its TATA region, the ability of a fragment to depress transcription is correlated with its ability to function as a transcriptional terminator (B. Osborne and L. Guarante, personal communication).

Discussion

Our experiments provide genetic evidence that a bacterial repressor protein manufactured in the yeast cytoplasm can enter the yeast nucleus, recognize its operator and repress transcription from a yeast promoter. Immunofluorescence experiments (performed in collaboration with P. Silver) show that LexA protein is localized to the nucleus in a minority of cells in a population that produces it (data not shown). In most members of the population, fluorescence is uniformly spread throughout the cytoplasm and we conclude that LexA protein has passively entered the nucleus. This behaviour is in contrast to the strictly nuclear localization of regulatory proteins native to yeast, which are thought to have a distinctive stretch of amino acids causing them to enter and remain in the nucleus (refs 13, 14, R. Moreland and L. Hereford, personal communication). Uniform cellular distribution of LexA protein should be sufficient to repress operator-containing *GAL1* promoters. If LexA protein constitutes 0.025% of the total protein in cells grown on galactose, and if it is evenly distributed throughout the cell, there should be about 750 LexA molecules in the nucleus. As the volumes of *E. coli* and a yeast nucleus are about the same, nuclear LexA protein concentration is probably equivalent to the concentration found in a single *E. coli*, which contains 100–1,000 molecules of LexA protein (unpublished data). We have shown here that the affinity of the synthetic operator for LexA protein is at least as high as that found for naturally occurring *lexA* operators. Therefore, 100–1,000 molecules of LexA per nucleus should be sufficient to occupy the operator¹⁵.

Consider two possible mechanisms by which LexA protein might repress *GAL1* transcription. First, LexA protein could repress *GAL1* transcription because its binding site is close to or overlaps the binding site of some protein necessary for transcription. Although some sites of operator insertion are located far from either the upstream activator sequence or the TATA region, all sites are at least moderately close (within 59 nucleotides) to one region or the other. Therefore, we cannot exclude this possibility. Note, however, that LexA protein has no effect at a site 28 nucleotides upstream of the upstream activator sequence, so proximity of an operator to the upstream activator sequence is not sufficient to produce a repressible promoter.

Second, the observed repression may be caused by LexA protein repressing *GAL1* transcription by blocking transmission of some stimulus (for example, RNA polymerase or some component of the transcription apparatus) from the upstream activator sequence to the TATA region. These two mechanisms are not mutually exclusive; depending on the location of the *lexA* operators within the *GAL1* promoter, one or both of them may exist.

Our results with transcription terminators reported here might be regarded as evidence in support of the second proposed mechanism. Transcription between the upstream activator sequence and the TATA region, however, has never been observed. It is possible that RNA polymerase makes an unstable transcript between the upstream activator sequence and the observed transcription start point, or that RNA polymerase or some other component of the transcription apparatus moves through this region in some other manner sensitive to the effects of transcription terminators. But we cannot exclude the possibility that a protein binds to the functional terminator but not to the mutant terminator, and that the bound protein interferes with transcription in the same way as LexA protein.

There is evidence that other yeast genes can be repressed by negative regulatory factors bound between the upstream activator sequence and the TATA region. For example, it is likely that *MATA1* transcription is repressed in diploid yeast by binding of a negative regulatory factor (which is dependent on the expression of *MATA1* and *MAT α 2*) to a site between the *MATA1* upstream activator sequence and its TATA region¹⁶. *STE6* transcription is repressed by the *MAT α 2* product, which binds to a specific site in the *STE6* promoter, probably located between the upstream activator sequence and the TATA region (A. Johnson, K. Wilson and I. Herskowitz, personal communication). The repression of *MATA1* and *STE6* is at least 50 times greater than the 10-fold repression we observe for *lexA* operator-containing derivatives of the *GAL1* promoter. We do not understand why these yeast promoters are repressed so efficiently relative to the *lexA* operator-containing *GAL1* promoter.

Further studies may use well-characterized prokaryotic regulatory proteins to control expression of other yeast genes and genes in higher eukaryotes. It will be of interest to see whether repressor proteins can block the activation of genes by enhancer sequences¹⁷ found in higher eukaryotes. Repressor proteins may also be useful in studying the mechanism of negative regulation in yeast in those cases where negative control is exerted at a distance, such as the repression of silent genes of the yeast mating type locus by the *MAR/SIR* gene products¹⁸.

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Primary structure and genomic organization of the histidine-rich protein of the malaria parasite *Plasmodium lophurae*

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The nucleotide sequence of a complete genomic clone for the histidine-rich protein of Plasmodium lophurae has been determined. The deduced amino acid sequence of the mature protein shows numerous tandemly repeated units preceded by a signal and a pro peptide. The gene is interrupted by an intron with a separate exon coding for the signal peptide. The signal peptide-encoding exon detects multiple cross-hybridizing sequences in the parasite genome.

THE protozoan parasite *Plasmodium lophurae* causes malaria in birds, invading host erythrocytes via a mechanism which involves specialized intracellular parasite organelles and surface receptors on both the parasite and the erythrocyte¹⁻⁴. Recognition and binding of the two cells is followed by complete engulfment of the protozoan cell by the erythrocyte, whereupon the parasite undergoes several rounds of asexual divisions. Mature daughter cells escape from their intraerythrocytic confinement to begin a new round of erythrocyte invasion.

In the intraerythrocytic stages of development of *P. lophurae* in ducks, there is synthesis of a major protein that accumulates to comprise at least 50% of the cellular mass. This protein, a basic polypeptide of relative molecular mass (M_r) 45,000 comprising 73% histidine, is located in a membrane-bounded compartment that forms part of the specialized parasite organelles implicated in erythrocyte invasion⁵. The function of the protein is unknown. In one series of experiments, antibodies to this protein were found to be protective⁶, but other investigators have been unable to reproduce these results^{7,8}.

We have shown that the early biosynthetic forms of the histidine-rich protein resemble those of secretory proteins⁹. Translation of parasite mRNA in a cell-free wheat-germ translation system yielded a larger precursor that was translocated into dog pancreas microsomal membrane vesicles. The segregated form was larger than the mature protein and contained Asn-linked oligosaccharide⁹. These data suggested that the histidine-rich protein is synthesized as the prepro-protein containing two transient sequences, a pre-sequence that functions as a signal sequence for translocation across the rough endoplasmic reticulum, and a glycosylated pro-sequence of unknown function.

We have now isolated a genomic clone that contains the entire histidine-rich protein gene and have determined its DNA sequence. The gene is encoded in two exons, separating the signal peptide-encoding sequence from the pro-sequence, confirming that synthesis of the protein occurs via the prepro-protein. Oligonucleotide probes synthesized to the signal peptide-encoding exon reveal multiple homologous DNA sequences in the *P. lophurae* genome. The sequence of mature protein is arranged in numerous tandem repeats with up to nine histidine residues in a row, similar to other *Plasmodium* proteins for which sequence data have so far been reported^{10,11,36-39}.

Genomic clone isolation

A partial cDNA clone, obtained by screening a *P. lophurae* cDNA library with a synthetic oligonucleotide encoding a poly-histidine sequence (ref. 12 and R. Feder *et al.*, in preparation), was used to determine the genomic organization of the histidine-rich protein. *P. lophurae* DNA digested with *Hind*III (Fig. 1a, lane 3) or *Eco*RI (Fig. 1a, lane 1) and probed with a histidine-rich protein cDNA probe detected a 7.7-kilobase (kb) and 1.8-kb fragment, respectively. The 7.7-kb *Hind*III fragment was cloned

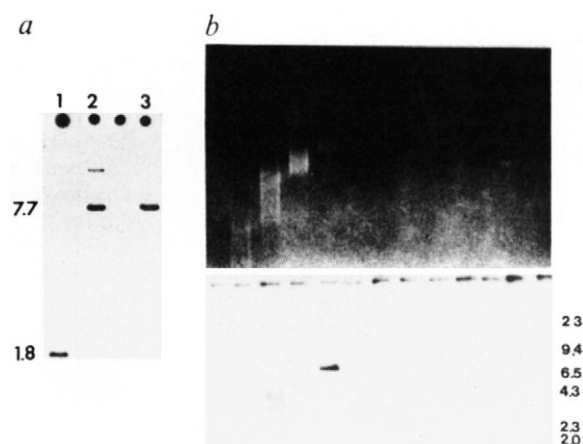


Fig. 1 *a*, Genomic cloning of the gene for histidine-rich protein into λ L47.1. *b*, Preparative agarose gel electrophoresis of *P. lophurae* DNA to enrich for the 7.7-kb *Hind*III fragment containing the gene.

Methods: *a*, *P. lophurae* was maintained in infected ducklings²⁶. DNA was isolated from infected duck erythrocytes by the saponin lysis procedure^{27,28}. 2 μ g of high-molecular weight DNA were digested with *Eco*RI (*a*, lane 1) or *Hind*III (*a*, lane 3) and the resulting fragments were separated on a 0.75% agarose gel and transferred to nitrocellulose paper²⁹. The gel was probed with nick-translated histidine-rich protein cDNA labelled to a specific activity of 2×10^8 c.p.m. μ g⁻¹ and hybridized in 50% formamide, 10% dextran sulphate, $5 \times$ SSC, $1 \times$ Denhardt's, 200 μ g ml⁻¹ of salmon sperm DNA at 40 °C for 16 h. Nonspecific hybridization was washed off in $0.1 \times$ SSC, 0.1% SDS at 54 °C and the filters exposed to Kodak XAR film with Dupont Lightening Plus intensifying screens at -70 °C for 4-16 h. The specific 1.8-kb *Eco*RI and 7.7-kb *Hind*III fragments which hybridize to the cDNA probe are indicated. *a*, Lane 2: DNA isolated from clone 8A, digested with *Hind*III and co-electrophoresed with *P. lophurae* DNA to demonstrate that an intact fragment had been cloned. *b*, 100 μ g of *P. lophurae* DNA were digested to completion with *Hind*III and fractionated on a Bulls Eye Electrophoresis apparatus (Hoefer Scientific). Fractions were collected, and aliquots analysed on a 0.75% agarose gel (*b*, upper panel). The DNA fragments were transferred to nitrocellulose paper and probed with the cDNA probe. DNA fragment sizes are indicated in kb. A peak fraction containing the 7.7-kb *Hind*III fragment is visible in the lower panel. This fraction was ligated into λ L47.1 *Hind*III arms, packaged *in vitro*³⁰ and used to infect LE 392. 1×10^5 recombinant phage were obtained from a microgram of *P. lophurae* DNA. 5×10^4 phage were screened by *in situ* hybridization³¹ with the nick-translated cDNA probe; a positive obtained, referred to as 8A, was plaque-purified and DNA isolated from this phage was mapped against *P. lophurae* DNA (*a*, lane 2).

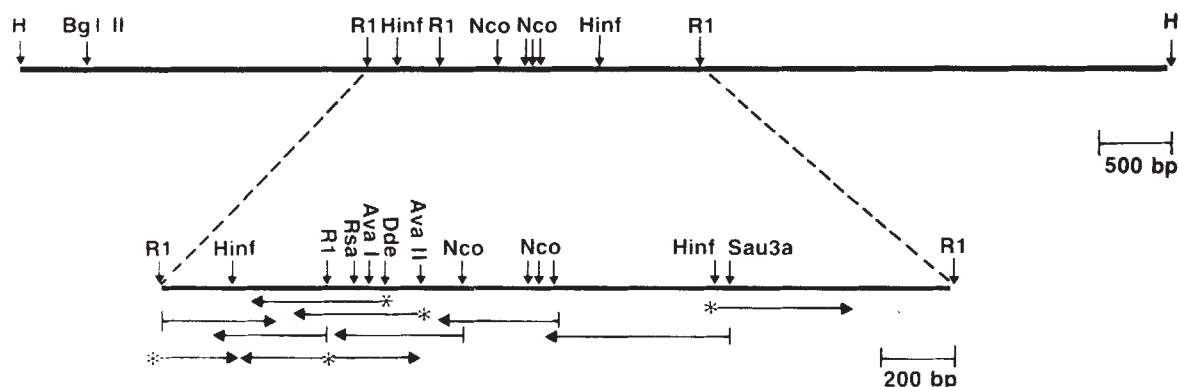


Fig. 2 Restriction map analysis and sequencing strategy for clone 8A of the histidine-rich protein gene. The 7.7-kb *Hind*III fragment, cloned as described in Fig. 1 legend, was mapped both within the phage and from a pBR322 subclone. Fragment sizes of the cloned DNA were compared with *P. lophurae* DNA. DNA sequencing analysis was performed using both the dideoxy method of Sanger and Coulson³², indicated by arrows ending with vertical lines, and by the chemical method of Maxam and Gilbert³³, indicated by arrows ending with stars. Fragments were obtained both from the phage clone 8A and from the pBR322 subclone. Repeat sequences obtained with the same fragment or M13 clone are not shown. The sequence obtained from the 3' *Hinf* site to the 5' *Nco* site was derived from two M13 clones which could be obtained in only one orientation due to the instability of the sequences in *Escherichia coli*. Multiple independent isolates of these clones were sequenced to generate the data shown in Fig. 3. This overlap, however, may be subject to some ambiguity.

ments by enriching *Hind*III-digested *P. lophurae* DNA by preparative agarose electrophoresis (Fig. 1b). Screening of 50,000 recombinant phages with the cDNA probe yielded a positive clone identified as 8A. Propagation of 8A in the bacterial vector LE 392 resulted in spontaneous deletion of the cloned insert, leading to segregation of the phage into two populations separable on CsCl density gradients. Only higher density, full-length phage particles were used for subsequent studies. *Hind*III digestion of clone 8A (Fig. 1a, lane 2) yielded a 7.7-kb fragment which co-migrated with the genomic *Hind*III fragment (Fig. 1a, lane 3). No deletion was apparent in this higher density phage fraction. The 7.7-kb *Hind*III fragment containing the genomic sequence of the protein was subcloned into pBR322 and propagated in HB101. Spontaneous deletion of the insert occurred in this system as well. A subclone, 8A-1, showed a 3.0-kb deletion in the 3' *Eco*RI-*Hind*III fragment of clone 8A (Fig. 2). *Eco*RI digests of clone 8A, 8A-1 and *P. lophurae* DNA revealed a co-migrating 1.8-kb fragment when probed with the cDNA probe (data not shown). *Bgl*II-*Nco*I digests of these DNAs revealed co-migrating 2.85-kb and 200-base pair (bp) fragments when probed with the 1.8-kb *Eco*RI fragment. Similarly, *Hinf*I digests of these DNAs revealed a common 1.35-kb fragment when probed with the 1.8-kb *Eco*RI fragment. These data confirm that no deletion or rearrangement had occurred in the 7.7-kb *Hind*III fragment in clone 8A or in the sequences extending from the *Bgl*II site 5' of the gene to the *Eco*RI site 3' of the gene in the subclone 8A-1.

Gene structure

The DNA sequencing strategy for the region encoding the histidine-rich protein (Fig. 2) revealed a sequence of 1,648 nucleotides (Fig. 3). An open reading frame coding for 328 amino acids (225 are histidine) extends from nucleotide 491 to nucleotide 1,487. Starting at nucleotide 563, we find a sequence identical to the recently reported sequence of 25 NH₂-terminal residues of mature histidine-rich protein¹³. There was no methionine in our reading frame, however, suggesting that the gene is interrupted and that the amino-terminal portion of the protein is located on another exon. Further analysis revealed another open reading frame with a putative initiation codon located at nucleotides 291-293, ending in a stop codon (nucleotide 366-368), and immediately preceded by a splice sequence AAGCGTAAG (boxed in Fig. 3) similar to the consensus 5' splice sequence, AAGGTAAG¹⁴. Similarly, a 3' splice sequence, TTATAG (boxed in Fig. 3), similar to the consensus 3' splice sequence, TTXCAG¹⁴, is found immediately adjacent to the next exon. We designed experiments to identify the predicted intron.

To demonstrate that the genomic DNA is not contiguous with its mRNA, we performed S₁ nuclease mapping. A 427-bp *Ava*I-*Hinf*I fragment (Fig. 4) spanning the intron-exon border was 5' end-labelled on the noncoding strand. After strand separation, the 5'-labelled strand was annealed to *P. lophurae* RNA. S₁ nuclease treatment of this hybrid yielded a 130-bp protected fragment (Fig. 5a, lane 2), consistent with a discontinuity between the RNA and the genomic DNA at nucleotide 491, at the intron-exon border.

To identify the break point between the DNA and RNA sequences specifically, we carried out primer extension studies. A 42-bp *Ava*I-*Rsa* fragment (Fig. 4) was labelled on the 5' end of the noncoding strand. After strand separation, the 5'-labelled strand was annealed to *P. lophurae* RNA. A cDNA was synthesized in the presence of dXTPs and reverse transcriptase and the resulting 600-bp fragment was isolated and sequenced by chemical degradation. Parallel sequences were obtained from a 427-bp *Ava*I-*Hinf* fragment (Fig. 4) labelled at the *Ava*I 5' end. The primer-extended sequence (Fig. 5b, lane 2) diverges from the genomic sequence (Fig. 5b, lane 1) at the position of the putative intron (arrow, Fig. 5b). The sequence derived by primer extension (Fig. 5c) agrees precisely with the 5' exon sequence (Fig. 3). A 5'-untranslated sequence can be read from the primer extension experiment for at least 150 nucleotides 5' of the exon, which similarly is in agreement with the genomic sequence. Additional S₁ mapping studies (data not shown) demonstrate that the 5'-untranslated sequence extends for at least 300 nucleotides beyond the open reading frame for the signal peptide. The precise 5' end of the untranslated mRNA sequence has not been identified. R-loop mapping of the genomic clone and mRNA (data not shown) demonstrates a 1,400 nucleotide R-loop, corresponding to the histidine-rich exon and 3'-untranslated sequences. Therefore, the length of the 3'-untranslated sequence is deduced to be 400 nucleotides (Fig. 4). The size of the mRNA has been identified by Northern gel analysis to be 2,200 nucleotides¹², suggesting that the 5'-untranslated sequence is approximately 700 nucleotides long.

Amino acid sequence

The prepro-protein contains 351 amino acid residues and, in its unglycosylated form, has a calculated *M_r* of 49,000. The amino acid sequence is numbered beginning at -47 for the signal peptide, at -24 for the pro-peptide and at +1 for the mature protein.

The assignment of the methionine at -47 as the initiating methionine of the prepro-protein needs to be confirmed by amino acid sequencing of the primary translation product. Our principal argument in support of this assignment is that the 24

Two synthetic oligodeoxynucleotide probes comprising the 5' exon were synthesized. These oligonucleotides were 5' end-labelled and used as probes to determine the genomic organiz-

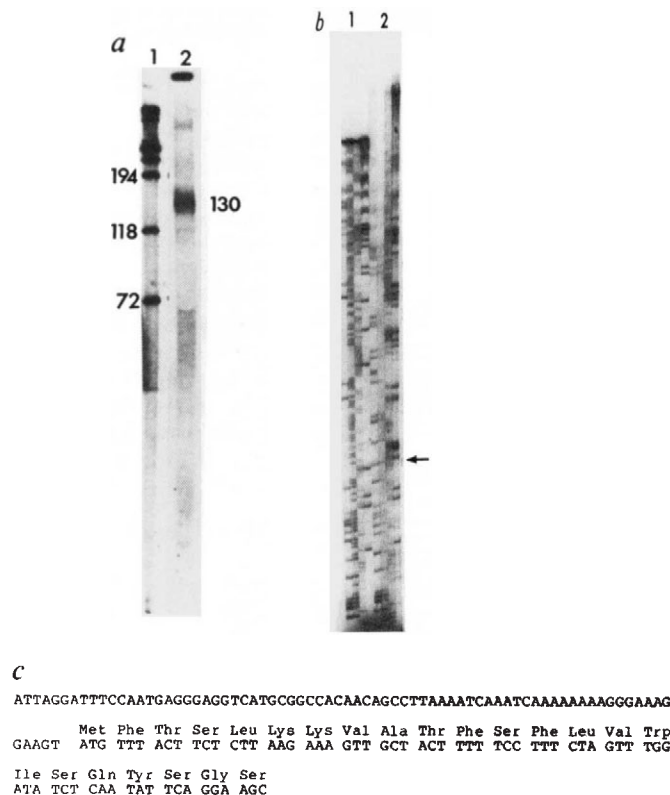
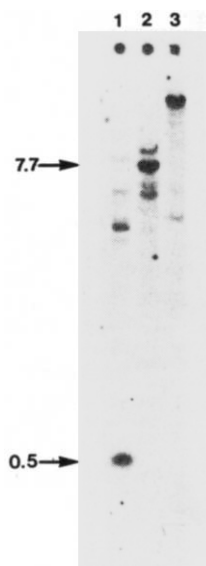


Fig. 5 Mapping the intron in the gene for histidine-rich protein.

a, S_1 nuclease mapping; **b**, primer extension. Lane 1, an autoradiogram of the DNA sequencing gel for the genomic *AvaI*-*HinfI* fragment; lane 2, the primer-extended fragment. **c**, cDNA sequence of the 5' exon and untranslated region deduced from the primer-extension experiment in **b**. The predicted amino acid sequence is shown above the nucleotide sequence, identical to the genomic sequence in Fig. 3. The sequence beyond the break point is shown, corresponding to the 5' exon sequence. (Identical 3' sequences for the two fragments are not shown.)

Methods: **a**, A 427-bp *AvaI*-*HinfI* fragment, spanning the intron-exon border, was isolated (see Fig. 4), labelled on the 5' *AvaI* end and strand-separated on a 10% polyacrylamide gel²⁴, 2×10^4 c.p.m. were co-precipitated with 10 μ g of *P. lophurae* mRNA in 70% ethanol at -20°C for 16 h. The precipitate was resuspended in 30 μ l of 80% formamide, 0.4 M NaCl, 40 mM PIPES, pH 6.4 and 1 mM EDTA. The reaction was incubated at 80°C for 15 min, rapidly cooled to 50°C , then incubated at 50°C for 3 h. The reaction was diluted with 0.3 ml of S_1 buffer (0.28 M NaCl, 0.05 M NaAc, pH 4.5, 4.5 mM ZnSO_4) and 300 units of nuclease S_1 were added. The reaction was incubated for 30 min at 37°C and stopped by adding 10 μ l 0.5 M EDTA. After phenol-chloroform extraction, the S_1 nuclease-resistant material was ethanol-precipitated, resuspended in 95% formamide and dyes and fractionated on a 10% acrylamide, 7 M urea sequencing gel. The gel was dried and autoradiographed for 16 h. Size markers (lane 1) are indicated, as is the position of the protected DNA fragment at 130 nucleotides (lane 2). **b**, A 42-bp *AvaI*-*Rsa* fragment was labelled on the *AvaI* end, strand-separated and coprecipitated with 10 μ g of *P. lophurae* mRNA in 70% ethanol at -20°C for 16 h. The pellet was resuspended in 50 μ l of 70% formamide, 1 mM EDTA, 0.5 M NaCl, 40 mM PIPES, pH 6.4 and incubated for 16 h at 56°C . The hybridized material was collected by ethanol precipitation and resuspended in 50 μ l of 0.1 M NaCl, 1 mM EDTA, 10 mM Tris, pH 8.3. An extension reaction of 100 μ l volume containing 50 mM Tris pH 8.3, 10 mM MgCl_2 , 1 mM of each of the four dNTPs, 10 mM dithiothreitol and 100 units of reverse transcriptase was incubated at 42°C for 1 h and terminated by the addition of EDTA to 10 mM, followed by phenol extraction and ethanol precipitation. The pellet was resuspended in formamide-dye loading buffer and fractionated on a 10% DNA sequencing gel. The resulting 600-nucleotide fragment was eluted from the gel and subjected to DNA sequencing by the method of Maxam and Gilbert²³. The arrow indicates the break point of the two sequences, which corresponds to the intro-exon junction (see Fig. 3).

Fig. 6 Multiple occurrence of the signal exon sequence in the *P. lophurae* genome. Two synthetic oligodeoxynucleotides with the sequences 5' CCAACTAGAAAGGAAAAAGTAGC 3' (complementary to nucleotides 315-338) and 5' AACTTCTTAAGAGAAGTAAACAT 3' (complementary to nucleotides 291-314) which correspond to the signal exon sequence (see Fig. 3) were synthesized, 5' end-labelled to a specific activity of 1×10^8 c.p.m. μg^{-1} and purified on a 15% acrylamide, 7 M urea gel. DNA from *P. lophurae* was digested with *EcoRI* (lane 1), *HindIII* (lane 2) and *BglII* (lane 3), transferred to nitrocellulose and hybridized with 10^7 c.p.m. of the oligonucleotide probes at 50°C in $6 \times \text{SSC}$, 0.1% SDS, $10 \times \text{Denhardt's}$ and 200 mg ml^{-1} salmon sperm DNA for 16 h. The filter was washed twice at 25°C in $6 \times \text{SSC}$, 0.1% SDS and twice at 37°C in $6 \times \text{SSC}$, 0.1% SDS. The filter was dried and exposed to Kodak XAR film at -70°C with an intensifying screen for 16 h. The 0.5-kb *EcoRI* and 7.7-kb *HindIII* gene fragments are indicated.



ation of the 5' exon in the *P. lophurae* genome (Fig. 6). In addition to the expected 0.5-kb *EcoRI* fragment and the 7.7-kb *HindIII* fragment (see Figs 1 and 2), multiple DNA fragments were detected with the enzymes chosen to digest the *P. lophurae* DNA, whereas only a single DNA fragment hybridized to the cDNA (see Fig. 1), indicating that the 5' exon sequence is present in multiple copies in the genome. Hybridization at reduced stringency using the 1.8-kb *EcoRI* fragment failed to detect additional cross-hybridizing sequences (data not shown).

To determine whether any of these cross-hybridizing sequences are closely linked to the gene for histidine-rich protein, clone 8A and subclone 8A-1 were digested with multiple restriction endonucleases; no additional DNA fragments hybridized with the oligonucleotide probes (data not shown), establishing that >2.0 kb must separate the signal peptide exon from the cross-hybridizing sequences.

Conclusions

Tandem repeats have been reported for other *Plasmodium* proteins in which the amino acid sequences derived from their cDNA and genomic clones reveal only one type of repeat. It remains to be seen whether these proteins, like histidine-rich protein, show several distinct repeats. The sequences of the repeats differ in all these proteins.

The surface antigens of *Plasmodium falciparum* may be a family of molecules comprised of repeating, antigenically distinct subunits¹¹. If histidine-rich protein becomes a surface molecule of *P. lophurae*, it must be secreted. Indeed, its early biosynthetic forms resemble those of secretory proteins^{19,20}, but it has not yet been established whether, and at what point of parasite development, secretion of the protein occurs. If secretion does occur, it would be followed by disassembly of the protein aggregates (perhaps by partial proteolysis) before association with the parasite surface.

The 131-nucleotide intron separating the signal peptide-encoding exon from the exon coding for histidine-rich protein demonstrates highly conserved splice sequences. Of interest is the occurrence of a sequence element at nucleotides 458-466 (TCTTTAACA), 22 nucleotides upstream of the 3' splice site, which is very similar to the splicing signal sequence in yeast (TACTAACA)^{21,22}. This TACTAACA box has been found 20-55 nucleotides upstream of the 3' splice site in all split protein-coding nuclear genes of *Saccharomyces cerevisiae* and is an essential element of the yeast splicing mechanism. These data suggest that *Plasmodium* possesses the cellular machinery to process precursor RNAs into mature message, perhaps using a mechanism similar to that of yeast.

We have shown here that the sequence comprising the signal peptide-encoding exon is present in multiple copies in the *P. lophurae* genome. Thus far, the conservation of a signal sequence

at the DNA level is unique to *P. lophurae* and without precedent in other genomes. This finding is particularly interesting when viewed in the context of a 35-bp long sequence that occurs in a tandem array in the genome of trypanosomes. This sequence can be translocated and is found associated with mRNAs coding for variable surface glycoproteins²³⁻²⁵ and other cellular mRNAs³⁵. This 35-bp sequence of unknown function occurs at the 5'-untranslated end of these mRNAs. Our data, presented here, demonstrate the redundancy of a 5' exon for another protozoan and suggest that a coding sequence of known function may be used by a diverse population of genes. Investigations are under way to identify these signal peptide-encoding exons

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in the plasmodial genome and to assign the exons to expressed mRNAs.

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LETTERS TO NATURE

A runaway instability in thick accretion disks?

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Others have proposed¹ that thick disks of material accreting around black holes may be subject to a runaway instability. If the mass of the disk exceeds a critical value ($m_{\text{disk}} > 0.01 M_{\text{hole}}$), the disk drains into the hole on a dynamical time scale. We report here a study of this effect, using models of non-self-gravitating disks in the Kerr metric, which enables proper allowance to be made for the effects of black-hole rotation and the increase in the spin of the hole as it accretes. We conclude that there is no runaway instability in these systems.

The construction of disk models in the standard approach, due to the school of Abramowicz, is straightforward^{2,3}. For an axisymmetric stationary space-time, the metric has the form⁴

$$ds^2 = -e^{2\nu} dt^2 + r^2 B^2 \sin^2 \theta e^{-2\nu} (d\varphi - \omega dt)^2 + e^{2\mu} (dr^2 + r^2 d\theta^2) \quad (1)$$

where ν , μ , ω and B are functions of polar coordinates r and θ . If the disk has negligible influence, equation (1) will be the Kerr metric. The specific angular momentum of the disk material, l , is given an assumed distribution; but here l is taken to be constant, for reasons discussed below. The disk material is assumed to be barytropic; here it has the equation of state of an isentropic radiation-dominated plasma, with $p \propto \rho^{4/3}$ (ρ is the rest-mass density, and the total mass energy density is $\varepsilon = \rho + 3p$). However, these assumptions are not critical. By assuming only azimuthal motion and that l is constant, the hydrodynamic equations can be integrated to give²

$$\int_0^p \frac{dp}{\varepsilon + p} = W - W_0 = -\ln u_t / u_0 \quad (2)$$

where W is the combined gravitational and centrifugal potential and u_t is the appropriate component of the four-velocity, which

has a physical meaning as the specific 'energy at infinity' and is calculated from l and the metric². The quantities u_0 and W_0 are those evaluated at the inner edge of the disk. For the equation of state assumed here, equation (2) becomes

$$\rho = K \left(\frac{u_0}{u_t} - 1 \right)^3 \quad (3)$$

defining K as a parameter that depends (inversely) on the entropy. If K is large, the disk material is cool. The specific angular momentum, l , must lie between l_{ms} , the angular momentum of the innermost stable orbit, and l_{mb} , the angular momentum of the marginally-bound circular orbit. If $l \sim l_{\text{ms}}$, the disk is very small, whereas if $l \sim l_{\text{mb}}$ the disk is very thick.

The potential W has a cusp, due to relativistic effects, along a ring in the equatorial plane (see, for comparison, Fig. 1a of ref. 1). This cusp defines a critical equipotential surface (analogous to the Roche lobe in binary systems) within which a stable disk must be confined, otherwise material will flow into the hole. Using the Kerr metric and the integral for the mass of a rotating fluid configuration (equation (3.49) of Bardeen⁴), the mass of a non-self-gravitating disk that just fills its critical surface can be written as

$$m_{\text{disk}} = K m_c(M, J, l) \quad (4)$$

where m_c is a function of the hole mass M , its angular momentum J , and the specific angular momentum of the disk material l . Equation (4) can be solved by straightforward numerical integration.

Consider now what happens if a mass δm is transferred from the disk to the hole. The mass of the hole rises by δm , and, as the material has specific angular momentum l , the angular momentum of the hole rises by $l\delta m$. The metric around the hole changes and so does the four-velocity component u_t in the flow. If the accretion process is axisymmetric, we expect the specific angular momentum to remain constant. There are three flow variables in general relativity that reduce to the newtonian specific angular momentum in that limit: these are u_ϕ (four-velocity component), $l = -u_\phi/u_t$ and $j = (\varepsilon + p)u_\phi/\rho$. The equations of motion imply that if l is constant, then j is constant. In an axisymmetric flow, j is conserved along the flow⁵. Thus, if j is constant at the start, any axisymmetric accretion will leave

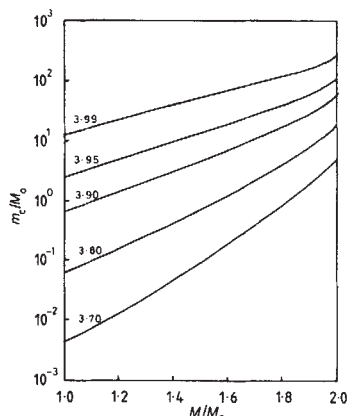


Fig. 1 Curves of the critical surface mass function, $m_c(M, J, l)$ as a function of increasing hole mass, M : m_c increases along the accretion trajectories defined by various values (given) of j/M_0 . ($J=0$ when $M=M_0$ —the initial mass.) As $dm_c/dM > 0$ everywhere, these curves show that all non-self-gravitating disks are stable with respect to the runaway instability.

j with the same value, independent of the mass-transfer process. This simplification is the reason for choosing l to be constant. During accretion, u_i decreases, implying that l increases, so the result of accretion is a disk with the same (constant) j and a larger l .

The runaway instability arises when a disk that just fits its critical surface loses a little of its matter to the hole. If the critical surface shrinks, the remaining matter may not fit in the surface, whereby more mass is lost to the hole, and so the process continues.

In the accretion process, unless energy is lost to infinity, the total gravitational mass of the system ($M_t = M + m_{\text{disk}}$) is constant. Consider a disk just filling its critical surface, for which $m_{\text{disk}} = m_c(M, J, l)$, that loses δm to the hole. The total mass of a system comprising a hole of increased mass and a critical surface-filling disk, with the same entropy (assumed constant in the process) and the same j , is $M'_t = M + \delta m + KM_c(M + \delta m, J + l\delta m, l + \delta l)$. If $M_t > M'_t$, then the disk does not fit the new critical surface and an instability arises. This condition can be shown to be identical to that at which the rest mass fits the critical surface. A set of M, j, l such that $dJ/dM = l$, and j is constant, defines the 'accretion trajectory' that a black hole and disk will follow, if mass is gained from the critical surface. Then the condition for stability along an accretion trajectory can be stated as

$$K \frac{dm_c}{dM} > -1 \quad (5)$$

The ranges of parameters that must be considered are $0 \leq J/M^2 \leq 1$ and $l_{\text{ms}} \leq l \leq l_{\text{mb}}$. It is found, throughout this parameter space, that $dm_c/dM > 0$, so all non-self-gravitating disks with constant l are stable with respect to the runaway instability. Figure 1 illustrates this in showing m_c always increasing along the accretion trajectories.

Why does our result differ from that of Abramowicz *et al.*¹? In simple terms, the size of the critical surface is governed by the ratio $(l - l_{\text{ms}})/(l_{\text{mb}} - l_{\text{ms}})$: when it is large, the disk is large. As the hole gains mass, l_{ms} tends to increase; if l is fixed, as it is in the pseudo-newtonian calculation, the ratio decreases, and the critical surface shrinks. The inclusion of relativistic effects changes this in two ways: first, as the hole accretes matter, it spins up, and a spinning hole has a lower l_{ms} than a non-spinning hole; second, l increases as the accretion proceeds. These two effects combine to make the ratio rise rather than fall when mass is transferred. Self-gravity is unlikely to affect this result.

A recent paper⁶ shows that constant angular momentum (newtonian) disks are subject to a non-axisymmetric instability. However, it is unclear whether such a disk is completely dis-

rupted, and whether the instability applies to disks with a significantly non-uniform distribution in l . Stable axisymmetric disks may exist and our result shows that these will not be subject to the runaway instability. Specific angular momentum, l , must increase outwards, so when material is lost from the inner edge of the disk to the hole, it is replaced by material with a higher value of l , which is more stable. Thus a disk with non-uniform l is less subject to runaway instability than one with uniform l , which, in turn, is stable with repeat to this process.

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Possible detection of a ring system in Saturn's magnetosphere

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The region of the magnetosphere of Saturn around 14 radii and 19 radii has been of special interest since the detection of anomalous dips in the low-energy plasma ion-density in these regions by the Voyager 1, Voyager 2 and Pioneer 11 spacecrafts. Lazarus *et al.* suggested that these density dips may be due to long-lived particulate or gaseous structures in the equatorial plane of Saturn¹. Baron and Elliot, using the charge-coupled device (CCD) imaging technique, found no evidence of any material existing between 10 and 36 Saturn radii². Their negative results, however, have set an upper limit on the extent of a possible material cloud with several assumptions regarding its nature and density. We have observed the occultation of the star SAO158913 from two stations in India following predictions by Mink³. This star showed symmetric dips in the light curves on 24 March 1984 (immersion event) and on 25 March 1984 (emersion event), suggesting the possible presence of particulate matter.

Figure 1 shows the path of the star relative to the planet. The outermost ellipse is the projection on the sky plane of a circular ring at 14 radii. The next inner ellipse represents the projection of the observed ring at ~12.5 radii. The visible inner rings are also shown for comparison.

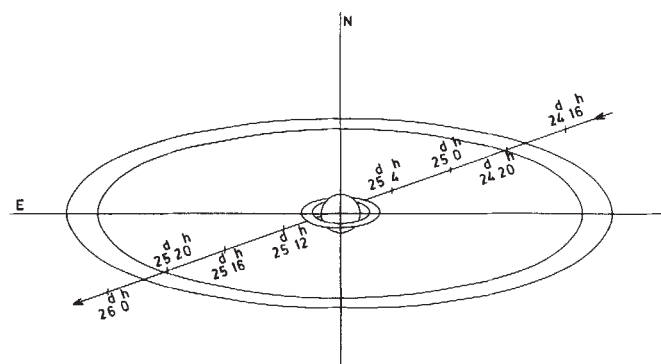


Fig. 1 Path of the star relative to the planet. The outermost ellipse is the projection on the sky plane of a circular ring at 14 radii. The next inner ellipse represents the projection of the observed ring at ~12.5 radii. The visible inner rings are also shown for comparison.

Observations were made on the 102 cm reflector at Kavalur⁴ (latitude +12° 34.58', longitude -5 h 15 min 19.6 s) using an EMI 9658 photomultiplier cooled with dry ice. Continuous d.c. recording (strip-chart recorder) and pulse counting using instrumentation developed locally were performed simultaneously on both nights. The continuous recording had a time constant of 1 s whereas a 5 s integration time was used in the photon counting mode. The observatory had photometric

observing conditions operating throughout 24 March and until 22 h 30 min UT on 25 March, as confirmed by steady photoelectric records taken over almost the entire duration. Observations on 24 March were made without a filter, whereas a standard B-filter [BG 12(4 mm) + BG 18(2 mm) + GG 4(1 mm)] was introduced on 25 March. A circular diaphragm of 12.4 arc s diameter was used on both nights.

The observations were made at night at Kavalur from 18 h 19 min to 21 h 25 min on 24 March, and from 20 h 3 min to 22 h 30 min on 25 March. On both nights the light curve was steady except for one brief interval each night lasting for ~42 min. Figure 2a and b shows the 5-s counts for these periods on the two nights after subtraction of the sky counts. The appearance of curve b has been reversed so that the structures of possible occulting material clouds can be easily compared on either side of the planet. Figure 3 shows the variation in star light as recorded by the strip-chart recorder. Gradual variation in intensity during immersion is observed between 19 h 55 min and 20 h 33.5 min; two sharp features appear between 20 h 14 min and 20 h 20 min. Similarly, during emersion, gradual variation in intensity is observed between 20 h 5 min and 20 h 45 min, and the sharp features appear between 20 h 18 min and 20 h 24 min. Besides these, several other extinction features of lower magnitude are apparent in these records. On the immersion side (24 March), the broad-band photometry indicates a maximum drop of ~0.77 magnitude, whereas on the emersion side (25 March), B-filter measurements show a maximum extinction of ~0.15 magnitude.

Additional observations on 24 March, 1984, at Uttar Pradesh State Observatory, Naini Tal (latitude +29° 21.65', longitude -5 h 17 min 49.71 s), were made with a 104 cm reflector using a standard V-filter and an EMI 6094 s photomultiplier (thermo-electrically cooled to -20 °C). The photomultiplier output was recorded on a strip-chart recorder. Figure 2c shows the strip-chart data digitized at intervals of 5 s. The maximum decrease in stellar flux is ~0.23 magnitudes. No observations could be made from Naini Tal on 25 March 1984 because of cloudy sky conditions.

Assuming that the observed extinction is caused by material orbiting the planet in the equatorial plane, we have calculated the planetocentric distances of the occulting material. Approximate positions of the two bodies have been extrapolated from data in ref. 3, which differ somewhat from those indicated by

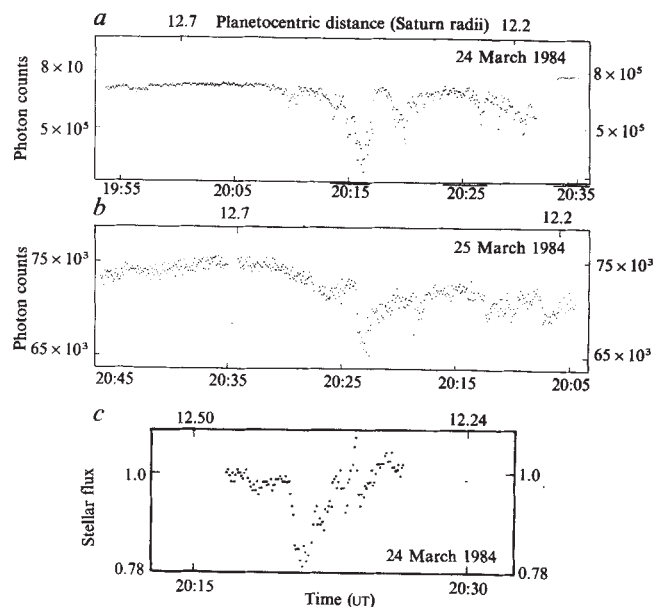


Fig. 2 Light curves: *a*, immersion on 24 March 1984 from Kavalur; *b*, emersion on 25 March 1984 from Kavalur, reversed for comparison with curve *a*; *c*, immersion on 24 March 1984 from Naini Tal. The Kavalur light curves were synthesized using the output of the photon counting unit with an integration time of 5 s. The normalized Naini Tal light curve was generated by digitizing the strip-chart record at 5-s intervals. The bottom and top scales along the abscissa give in each case the time in UT and the planetocentric distance in Saturn radii, respectively. Sky background has been subtracted in each case.

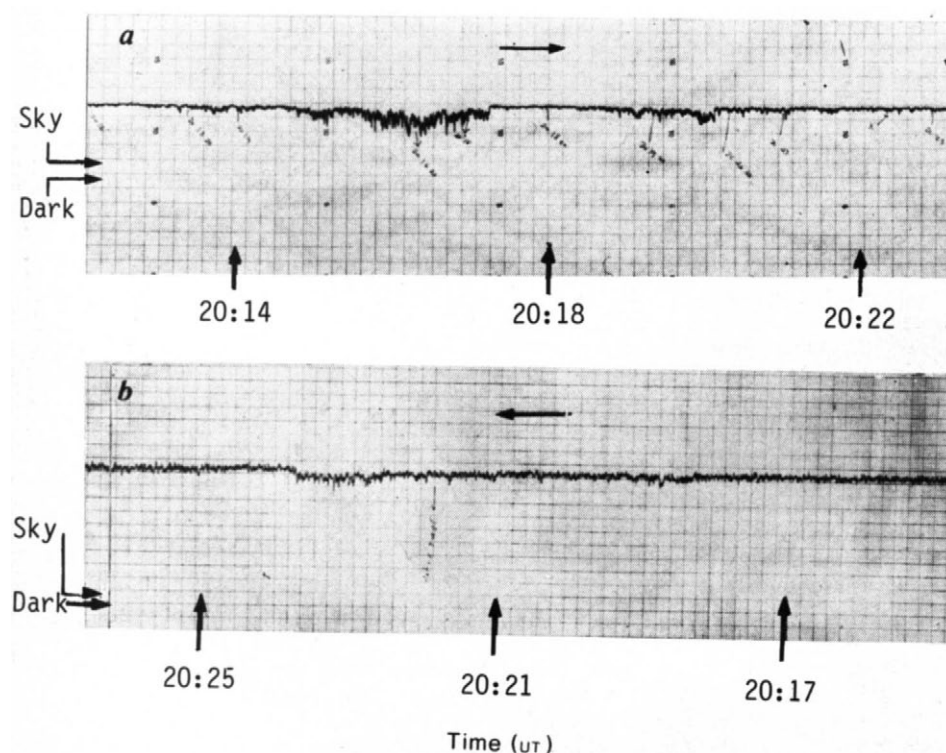


Fig. 3 Copy of the strip-chart record showing prominent features of the light curves obtained at Kavalur: *a*, immersion event on 24 March 1984 (20 h 12 min–20 h 23 min); *b*, emersion event on 25 March 1984 (20 h 26 min–20 h 14 min) reversed to allow direct comparison with the immersion light curve. The complete record is not shown (see text for description). Amplifier gains were different on the two days. Full scale corresponds to 10 mV.

the American Ephemeris record (1984) and the Smithsonian Astrophysical Observatory catalogue. More accurate values of the relative positions of the two bodies may become available when the times of A-ring occultations are determined. Hence our planetocentric distances are subject to modification by other events in the occultation not as yet taken into account. A typical error of 1.5" in declination and 0.1 s in right ascension, in either the star position or the planet's ephemerides, shifts the shadow centre of the planet by ~ 0.16 Saturn radii. Therefore, the calculated values for planetocentric distances may be in error by this amount.

The shapes of the main features of the three time profiles (Fig. 2) are similar, though the extinction magnitudes are different. The results indicate the probable existence of occulting material in orbit around Saturn at a planetocentric distance of ~ 12.5 Saturn radii. The planetocentric distances of the prominent features are not exactly the same in the three sets. This minor difference could arise from uncertainty in the relative positions of the star and the planet as mentioned above. The large discrepancy in the extinction magnitudes on the immersion side, as observed from the two sites, is puzzling, although the B-filter measurements on the emersion side almost agree with the V-filter observations on the immersion side. A realistic estimate of albedo from optical depth measurements depends on detailed modelling of the ring, which itself must await the results of further observations of occultation in other wavelength bands. An optical depth inferred from our observations would, in any case, generate an albedo of at least one or two orders of magnitude greater than the upper limit set by Baron and Elliot². This apparent inconsistency clearly emphasizes the necessity for more ground-based observations before further progress can be made.

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A 154-day periodicity in the occurrence of hard solar flares?

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Since the launch of the Solar Maximum Mission (SMM) satellite on 1980 February 14, the Gamma-Ray Spectrometer (GRS)¹ has been monitoring the Sun in the energy range 0.3–100 MeV. Because of its large geometric area and good spectral resolution, the GRS has provided unprecedented spectral and temporal information about solar flares, of which 139 have been monitored. We report here on an analysis of the temporal distribution of these high-energy events to provide information on solar activity and find that, instead of being randomly distributed in time, these events have a tendency to occur in groups with a mean spacing of ~ 154 days (75 nHz) over the observing interval. A larger sample of flares (>500), with an X-ray classification of $\geq M 2.5$ recorded by the GOES satellites, showed a similar regularity.

The SMM satellite was launched into a near-circular orbit with an inclination of 28.5° and an altitude of 570 km. The orbit precesses, relative to the Earth–Sun line, with a period of ~ 48 days. Because of the decay of the orbit, the period of revolution has decreased. In November 1980, the fine-pointing attitude

stabilization malfunctioned, halting operations of the solar imaging instruments, but adequate stabilization was maintained for the GRS and two other instruments using magnetic torquing bars. This failure did not affect the GRS measurements because of the large field-of-view of the detector ($\sim 2\pi$ sr). South Atlantic anomaly passages and the occultation by the Earth's orbit limit the duty cycle for the observation of solar events to $\sim 50\%$. Since the launch, the instrument has functioned properly with no evidence of degradation. In the period October 1983–mid-April 1984, after the repair mission, data recovery was sporadic, though normal operations are now continuing. This paper deals only with observations made from 1980 February 19 to 1983 September.

The search for energetic photon solar flares was performed by visual screening of the counting rates at ~ 300 keV, the low-energy threshold of the GRS, and by a computerized analysis of fluctuations in the 350–800-keV energy band². The solar origin of any transient event identified in this search was confirmed by solar observations made at other wavelengths³. Over the period from the launch to September 1983, we have identified 139 solar flares emitting continuum radiation of energy ≥ 300 keV. Figure 1 shows the temporal distribution of these flares. The flare frequency was rather high throughout 1982 despite the fact that the mean sunspot number had decreased by $\sim 30\%$ from its maximum⁴. In fact, the interval of greatest energetic photon flare activity occurred in June/July and November/December 1982, 2–3 years after the sunspot maximum for this cycle. A time delay with respect to the sunspot maximum was also found in previous solar cycles for solar energetic particle events (ground level effects)⁵. The frequency of hard flare events decreased dramatically in 1983, reflecting the continuous decrease in the relative sunspot number and other activity monitors such as the 10.7-cm radio flux.

We also note from Fig. 1 that the flares tend to occur in groups which are spaced by about 5 months. Eight groups are visible with their centres near 1980 June and November, 1981 April and September, 1982 February, July and December and 1983 May. We have assessed the quantitative significance of these apparent groupings by calculating a Fourier power spectrum of the time distribution of flares in which each is treated as an independent event irrespective of its parent active region (Fig. 2a). Several peaks are seen, the most significant (labelled A, B and C in Fig. 2a) being centred at 75, 155 and 227 nHz respectively, the latter two being the second and third harmonics of peak A. The subsidiary peaks around A, that is, A' and A'', are consistent with the power spectrum of a periodic time series truncated by the 1,185-day observation time interval. The full width of the primary peak A (~ 10 nHz) is somewhat wider than that expected for a 'pure' sine-wave time series truncated at 1,185 days (T) which would be $2/4T = 4.9$ nHz (ref. 6). The other peaks are not identified. Although it is not shown in Fig. 2a, we note that there is no significant power at a frequency of 429 nHz corresponding to the 27-day period of the solar rotation.

A detailed study of the time distribution of the flares and their active regions shows that two regions AR 3317 and AR 3804, were particularly prolific, producing 7 and 11 flares in 1981 and 1982, respectively. Each region produced their flares within a short time span (~ 10 days) and the time interval between the two regions was ~ 10 months. Removal of these 18 events from the time series resulted in a power spectrum which still had the most pronounced peak at 75 nHz, but with its peak power reduced by a factor of 1.5. Therefore, the events of these regions are not the sole cause of the strong peak in the power spectrum at 75 nHz. We have also calculated the power spectral density (PSD) using an independent fast Fourier-transform program⁶ on the same time series. In this case, all the strong features of Fig. 2a are reproduced and at the same frequencies. To estimate the significance level of the peak at 75 nHz, whose formal probability is $>6\sigma$ (ref. 7), four time series were run by this program, replacing the 139 observed events with an equal number of random ones within the same 1,185-day intervals. The PSD from random peaks in these test cases never exceeded

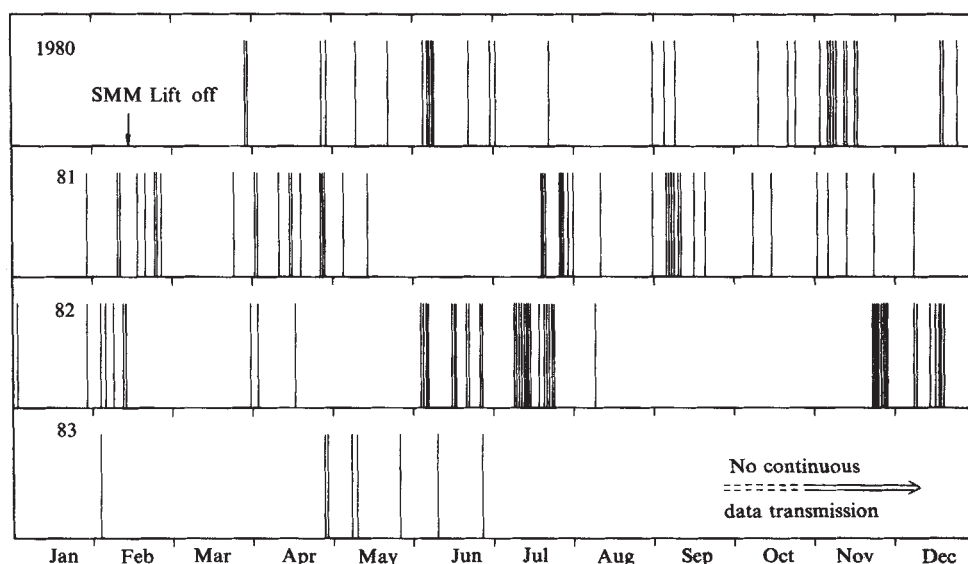


Fig. 1 Energetic solar flare events with photon emission of energy >300 keV are illustrated by short vertical lines, plotted against time for successive years of observation after the GRS began to operate.

$\sim 15\%$ of that for the real peak at 75 nHz, shown in Fig. 2a. This result confirms our formal estimate of the significance of the real peak: it is evident that this peak does not occur by chance and represents a strong 154-day recurrence tendency for energetic photon flares (>300 keV) during the time period of observations.

As already mentioned, we have to work with an incomplete data sample, because about half of the flares cannot be observed owing to the orbit of the SMM satellite. However, the data gaps introduced by the occultation and the close passages of the South Atlantic anomaly are of such a high frequency (170 μ Hz and 11.6 μ Hz, respectively) that they cannot be the cause of any artefacts around the main peak in Fig. 2a. The precessional motion of the orbit, which shows up as a slight change in the instrumental background ($\pm 10\%$) around 300 keV with a period of 48 days (~ 240 nHz), has a negligible influence on the detectability of flares. Finally, a Fourier analysis of 70 cosmic γ -ray bursts observed by GRS as a by-product has revealed no spectral feature around 75 nHz.

If we fold the flare-event times with the apparent period of 154 days, starting on 1980 May 26, and using a bin size of 15.4 days, we obtain the phase histogram shown in Fig. 3. Out of a total of 139 events, 48 (35%) occur within one 15.4-day bin. The broken line indicates the number of active regions which generated the flares in the respective time bin. To test whether the apparent nonuniformity of the latter distribution is statistically significant, we performed χ^2 -analysis. With nine degrees of freedom we obtain $\chi^2 \approx 42$, which corresponds to a probability of 10^{-5} that the time distribution of active regions was caused by chance.

Note also that a similar spectral feature at 75 nHz appears when a Fourier analysis is performed on all Hard X-Ray Burst Spectrometer (HXRBS) events, for which the counting rate was $>10^3$ s $^{-1}$, for photons >25 keV (ref. 8). In this case the total number of flares was 426 and they occurred in a time period overlapping that used in the present analysis and of a similar duration—1,342 as opposed to 1,185 days. As HXRBS is an experiment on the SMM satellite, this data sample also suffers from the fact that the Sun is visible only about 50% of the time. We have therefore analysed flares detected in soft X-rays by the GOES satellites with a GOES class of $\geq M 2.5$ registered during the same time interval³. Figure 2b shows the power spectrum for these 532 events. A prominent peak is seen at 76 nHz, corresponding to a period of 152 days. The difference in the period between the GRS events and the GOES events is insignificant if we take into account the width of the peaks. If we fold the GOES flare event times with the period of 152 days, 168 events (or 32% of the total sample) fall in a single phase bin of 15.2 days. Finally, we removed the 94 GRS flares from

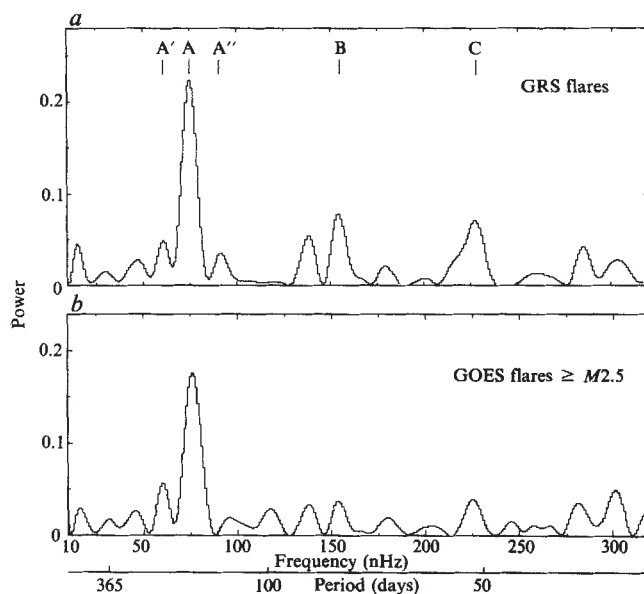


Fig. 2 a, Power spectrum of the time series shown in Fig. 1 (139 events). The individual peaks marked by letters are explained in the text. b, Power spectrum of GOES flares of $\geq M 2.5$ (532 events) which occurred during the same time interval as the GRS flares.

the GOES sample to test whether the regularity of the $\geq M 2.5$ events was caused by the regular occurrence of the GRS flares; but the power in the main peak was reduced by only 6%.

Is this regularity in the occurrence of X-ray flares a feature of cycle 21 which shows up also in other indicators of the solar activity? To check this we have plotted, in the upper part of Fig. 4, the monthly mean sunspot number together with the eight bursts in γ -ray flare activity (phase 0.4–0.5 of Fig. 3). We note also some periodic behaviour in the sunspot number, because the bursts numbered 3, 4, 5, 7 and 8 coincide closely in time with secondary sunspot maxima. For comparison we have also shown the declining phases of cycles 19 and 20 by arbitrarily aligning in time the absolute maxima of each cycle. Cycle 20 is different in appearance from the present one, because it is rather smooth, showing no indication of fluctuations which repeat at 5 months. Cycle 19, on the other hand, is more like cycle 21, because it has well developed secondary maxima, some of which follow in time intervals of ~ 5 months. Dodson and Hedeman⁹ found that increases in solar activity generally occurred with a repetition period of 3–5 rotations. Vitinskii¹⁰ found that the fluctuations in the relative sunspot number range

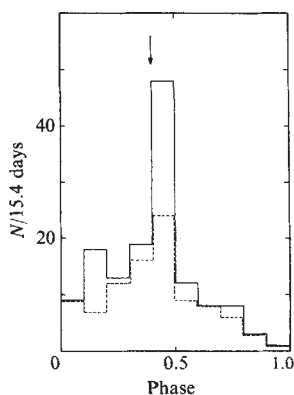


Fig. 3 Number of flares, N , per phase bin, folded by a period of 154 days (full line) and number of active regions contributing flares (dashed line). The absolute times of phase 0.4 (arrow) are: 1980 May 26; 1980 October 27; 1981 March 30; 1981 September 1; 1982 February 2; 1982 July 6; 1982 December 8; 1983 May 12.

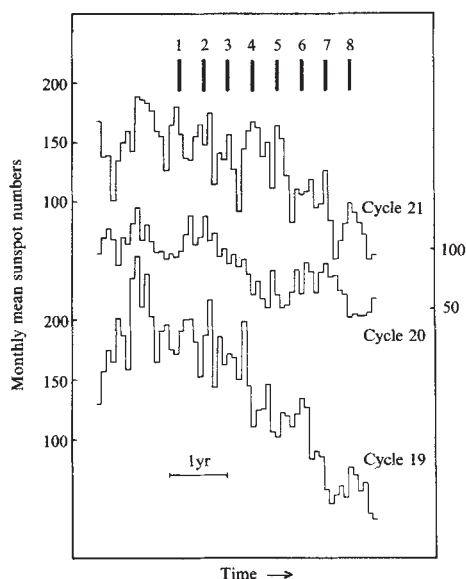


Fig. 4 Monthly mean unsmoothed sunspot number during the declining phases of cycles 19–21 (ref. 4). The cycles were aligned arbitrarily in time with their absolute maxima. The bars at the top of the figure mark the times with respect to cycle 21 when the eight bursts of high-energy flare activity occurred.

in time from 3 months to 1 yr, with an average duration of 5 months. The most extensive analysis of the monthly mean sunspot number was carried out by Wolff¹¹ who searched for significant frequencies using a 230-yr data base from 1749 to 1979 and found a set ranging from 15 to 180 nHz. He interprets these as the beat frequencies of the rotation rate of g-modes. (The g-modes are thought to be oscillations of the solar interior.) The most prominent period of <200 days found by Wolff is 155.4 days (74.49 nHz), which is close to what we have found for energetic solar flares.

Thus there is clear and compelling evidence for a 154-day regularity or periodicity in the occurrence of energetic flares. This effect was first recognized and measured for flares producing emissions of >300 keV and has been confirmed for flares producing soft X rays. The latter data set was obtained from multiple satellites having ~100% solar coverage and hence removes the possibility of any orbital selection effects. This regularity is not a minor effect involving only a few flares but, in fact, involves >30% of all the flares observed. The nature of this 154-day period is not yet understood but may have its origin in deeper layers of the Sun. If so, a better understanding will not only provide information on solar flare production but will

be an important probe of subphotospheric phenomena. It will be interesting to see whether the recurrence period observed for energetic photon flares (>300 keV) persists throughout the remainder of the SMM post-repair mission.

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Magnetic field amplification in the solar nebula through interaction with the T-Tauri wind

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As carbonaceous chondrites are the least thermally-evolved and hence the most primitive of the meteorites, their residual magnetization can, in principle, be used to estimate the intensity of the magnetic field in the primordial solar nebula, which varies between 0.2 and 1 G (refs 1–4), and could be as high as 2–3 G (ref. 4). The presence of palaeomagnetic fields of such magnitude is of importance in reconstructing the early history of the Solar System and of planetary formation. Levy and Sonnet⁵, for example, have stressed this point in comparing the respective merits of four alternative sources of the primordial magnetic field. They claim only two of these are possible: (1) a large solar magnetic field spread into the solar nebula; (2) a hydromagnetic dynamo field generated in the solar nebula itself. We show here that there is in fact a further possibility that fits the requirements for strong magnetic field generation and energetic particle irradiation of the grains⁶: magnetic field enhancement at the point of stagnation between the solar nebula and the intense solar outflows. This mechanism, which involves the interaction of the T-Tauri wind with the solar nebula, is straightforward and is supported by results from recent space research.

We argue basically that during the brief period ($\approx 10^5$ – 10^6 yr) of the T-Tauri phase of the proto-Sun, the solar nebula was being swept up by an intense solar wind with a mass-loss rate of $dM/dt \approx 10^{-7}$ – $10^{-6} M_{\odot} \text{ yr}^{-1}$ (refs 7, 8). This was accompanied by strong flare activities^{7,9} which contributed to the grain irradiation effect. However, the penetration of solar particles should at first be limited only to the inner edge and the boundary layers of the solar nebula (Fig. 1). This scenario is compatible with Wetherill's idea¹⁰ that, because of the blocking effect, the condensed grains forming Venus should be subjected to much more severe effects of primordial solar wind by implantation (^{20}Ne and ^{36}Ar , say) than those later forming the Earth. However, we require that the 'working surface' or the stagnation point between the T-Tauri wind and the inner edge of the solar nebula should progress outward to reach the condensation zone of the carbonaceous chondrites (that is, a solar distance $\approx 3 \text{ AU}$?).

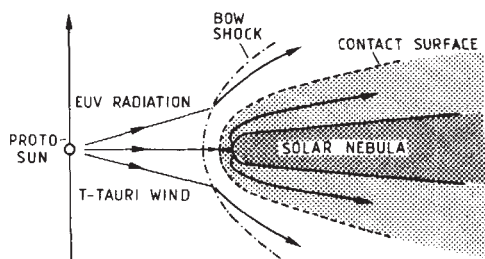


Fig. 1 A schematic view (not to scale) of the interaction process between the solar nebula and the T-Tauri wind of the early Sun. We postulated that, at the stagnation point between the solar wind and the solar nebula, the interplanetary magnetic field would be amplified to 0.1–2 G. Further leakage of the draped fields into the solar nebula would lead to magnetization of the condensed grains. EUV = extreme ultraviolet.

It is not known whether the solar nebula was clear of gas during the T-Tauri phase. In any event, even if there were little gas in the dust disk, the sputtering effect of the flare particles should be effective in generating a gas cloud. The subsequent ionization effect of the solar UV radiation and the charge-exchange process between the T-Tauri wind and the neutral gas will lead to a situation similar to that of solar wind interaction with comets, or Venus, in which the interplanetary magnetic fields are compressed (or draped) ahead of the ionospheres of these planetary bodies¹¹.

Interplanetary magnetic fields (flux $B_{\infty} \approx 1 \times 10^{-4}$ G), as demonstrated by the Pioneer Venus observations¹², are piled ahead of the contact surface separating the ionosphere of Venus and the shocked solar wind. The amplification effect can be approximated by equating the magnetic field pressure to the ram pressure of the solar wind. A similar relation has been used to estimate the interplanetary magnetic field piled ahead of a cometary ionosphere¹³, and detailed magnetohydrodynamic calculations^{14–16} have shown that the Lorentz force due to the radius of curvature of the captured field lines will be important, and will lead to further enhancement of the magnetic field. That is, if B_i is the peak field flux at the stagnation point, it could be given by

$$\frac{B_i^2}{8\pi} \approx (2-3)\rho_{\infty}V_{\infty}^2 \quad (1)$$

where ρ_{∞} and V_{∞} are the mass density and speed of the solar wind at large upstream distances, respectively. In the case of the T-Tauri wind, with $dM/dt \approx 10^{-7}$ – $10^{-6} M_{\odot} \text{ yr}^{-1}$, a wind speed (V) = 200 km s⁻¹, and a solar distance of ~ 3 AU from the proto-Sun, the application of equation (1) indicates that the magnetic fields in the vicinity of the working edge of the solar nebula could be amplified to ≈ 0.2 – 0.6 G even with a small seed field in the T-Tauri wind.

Note that the above description of magnetic field amplification in the vicinity of the stagnation point depends very much on the validity of treating the entire interaction process as continuous. It is possible also that kinetic effects might have been important in the actual situation, as argued previously for the case of comet-solar wind interactions¹⁷. But at least the observations at Venus, where these effects could certainly be said to be important in the solar wind interaction process, have indicated that equation (1) is not inappropriate.

In this connection, there is also an important difference between the azimuthally symmetric disk geometry of the solar nebula and those of comets and Venus. Most significantly, in the case of the solar nebula, the draped fields would not be moved away from the flanks by convection. In other words, whereas the stagnation points with enhanced magnetic fields are only localized regions in the vicinity of the hemispherical nose-cones of comets and Venus, for the solar nebula (if axisymmetrically shaped) it could form a belt-like structure extending 360° around the Sun. Without sideways-slippage, all the magnetic flux may tend to accumulate at the front. From this point of view, the maximum value of the draped fields could be

increased further beyond the prediction of equation (1). Whether it could reach 2–3 G, as obtained by setting the magnetic pressure equal to a thermal pressure of 10^{-6} bar in a limited region near the contact surface, must be resolved by future quantitative computations. Note that the continuous build-up of the magnetic fields at the front could be balanced only by continuous leakage into the inner region of the solar nebula or reconnection near the stagnation point. Both processes could take place: however, the leakage effect (by interchange instability or ambipolar diffusion) is more interesting as it allows an extensive portion of the solar nebula to be permeated by the draped magnetic fields with a magnitude of ~ 0.2 G. These fields would eventually be dissipated by magnetic diffusion or, once again, by magnetic reconnection at the equatorial current sheet.

We have extended the magnetic field amplification effect which has been observed in the solar wind-Venus interaction, and which is expected to occur in the solar wind-comet interaction, to the interaction of the dust and gas components of the primordial solar nebula with the T-Tauri wind of the proto-Sun. Using this extrapolation, strong magnetic fields (at least ≥ 0.1 G) could be generated at the stagnation point of the solar nebula and could even penetrate through the solar nebula to form a thin current sheet at the equatorial plane separating the two regions of opposite magnetic polarity. Whether or not a maximum field strength of 2–3 G could be generated, as inferred from the large permanent magnetization of the Allende meteorite, remains unclear. In any event, we believe that our proposed mechanism is promising compared with the alternatives reviewed by Levy and Sonett⁵ and they should be investigated further. We note that the present model may be considered as an important variant of the one involving spreading of the solar magnetic field into the solar nebula (proposal (1))². We expect in fact that a hydromagnetic dynamo field might be generated in the solar nebula (proposal (2))⁶ concurrently with the field-draping mechanism proposed here. However, the dynamo mechanism depends critically on the state of turbulence of the solar nebula, whereas the T-Tauri wind interaction scenario is relatively insensitive to the particular assumptions of turbulent velocity and scale-length of the turbulent eddies. Perhaps another point of interest is the azimuthal motion of the ionized matter in the vicinity of the contact point. In an idealized picture, such 'winding' of the draped fields should act to produce a toroidal component of the amplified field: either the draped field could be further enhanced by this process, or it could introduce complicated effects relating to the stability of the system which could be treated only in detailed numerical models. This particular issue, together with the questions of reconnection and leakage of magnetic fields, must await quantitative studies. Finally, in this connection, we note there is uncertainty about the timing between the T-Tauri phase of the proto-Sun and the condensation of the carbonaceous chondrites. However, the importance of the quasi-cometary interaction process as an element in the magnetohydrodynamics of the early solar nebula, as depicted here, is quite independent of these considerations.

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Observation of a thin layer of material in the upper stratosphere

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In this letter we report the results of laser radar measurements at Aberystwyth (52°25' N, 4°04' W) which revealed the presence of a discrete layer of atmospheric material of about 0.5 km thickness near 40 km for at least 3 h during the night of 4–5 July 1983. Wind data indicate that it was moving from the east and had an east–west extent of at least 170 km. We estimate that the layer had a mass content in excess of 100 kg for each degree of latitude width. This layer does not appear to be due to a natural terrestrial source or extraterrestrial material, and it seems likely that it was a result of pollution at upper stratospheric heights.

The observational system makes use of a Nd/Yag laser transmitting 300 mJ of energy at 530 nm in 15-ns pulses at a repetition rate of 10 Hz. The receiver includes a 1-m newtonian telescope fitted with a suitable interference filter and operated in a photon counting mode, the recording channels providing a height resolution of 60 m. The variations with height over the interval 36–42 km of the range-corrected backscattered signals recorded in successive 15-min periods between 22.05 UT on 4 July and 02.20 UT on 5 July 1983, are shown in Fig. 1. For clarity, the data are plotted as running means over 300 m, the standard errors for these means being presented at intervals of 1.5 km. The results for the first five 15-min periods show smooth height variations consistent with Rayleigh scattering. A small perturbation is shown near 39.5 km in the height variation shown for the sixth period, 23.20–23.35 UT on 4 July, and this feature becomes more pronounced with time, increasing to maximum amplitude in the fourteenth and fifteenth periods, 01.20–01.35 and 01.35–01.50 UT on 5 July, after which it seems to diminish. Unfortunately, observations were discontinued at the end of the seventeenth period at 02.20 UT. Measurements have been carried out on most clear nights since November 1982 with height resolutions of 300 m or better but no other example of such a thin layer has been observed. Note, however, that the greater degree of variability in the height variation of Rayleigh scattered signals observed on winter nights might obscure such a feature.

The change in magnitude and form of the perturbation with time is indicated in Fig. 2. This shows the mean data for periods 6–8 (23.20–00.05 UT), 9–12 (00.05–00.50 UT), 12–14 (00.50–01.35 UT) and 15–17 (01.35–02.20 UT) expressed in terms of the ratios R of the mean data for corresponding heights in the earlier periods 1–5 (22.05–23.20 UT). To provide representative measures of the peak height and thickness, running means over 120 m height are adopted. The values of $R-1$ represent the excess backscatter relative to the Rayleigh scatter from molecules at the corresponding height. The peak value approaches 10% for the periods 12–14. Based on the molecular densities from an atmospheric model for 45° N in midsummer¹, the integrated backscatter for these periods is estimated at $3.15 \pm 0.30 \times 10^{-7} \text{ sr}^{-1}$. If the scattering particles were composed of a sulphuric acid–water solution, as found at lower stratospheric heights, this value corresponds to an integrated mass of $5 \times 10^{-9} \text{ kg m}^{-2}$ (ref. 2).

Observations of dust layers in the upper stratosphere and lower mesosphere have previously been made from orbiting spacecraft², aircraft³, balloons⁴ and the ground^{5,7}. Most of these observations related to intermittent layers of thicknesses 10 km or greater at heights near 50 km or above. However, Clemesha and Nakamura⁷ reported laser radar observations of a layer of thickness 4 km, which dropped from 47 to 44 km within a day, and excess scattering over a range of heights during three subsequent months. They associated their observations with the influx of extraterrestrial material.

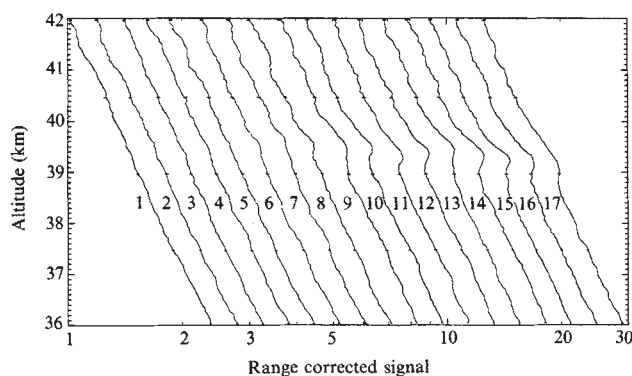


Fig. 1 Variations with height of the range-corrected signals at vertical incidence measured in 15-min intervals during 22.05 UT on 4 July to 02.20 UT on 5 July 1983; the plots for successive intervals are offset along the horizontal axis. Standard errors are shown at intervals of 1.5 km.

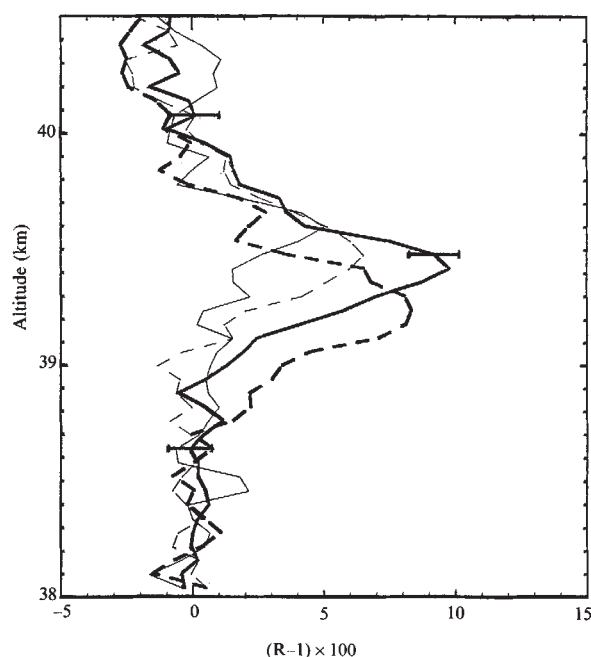


Fig. 2 Variations of the excess backscatter arising from the perturbation as deduced for four 45-min periods: — 23.20–00.05 UT; --- 00.05–00.50 UT; — 00.50–01.35 UT; --- 01.35–02.20 UT. R represents the ratio of the mean backscatter signal in these periods to the corresponding mean signals prior to the perturbation. Sample standard errors are shown.

The presence of aerosols, of variable concentrations, is a permanent feature of the lower stratosphere. However, measurements using several techniques, both ground-based and space-borne, have demonstrated that the material is generally confined to heights below 30 km (ref. 8). The violent eruption of the El Chichón volcano in Mexico in March–April 1982 did produce aerosols at heights extending up to 35 km during the first few months following the eruption^{9,10}, and observations at Aberystwyth recorded the presence of intermittent layers up to 34 km until the end of 1982. Subsequently, the enhanced aerosol concentrations were generally confined to heights below 30 km (ref. 11) and by June 1983 there was no evidence of aerosols at heights above 30 km. However, it is of interest to consider the El Chichón eruption as a possible source of the observed layer.

To be detected by scattering at 530 nm, the particles probably have radii of at least $0.1 \mu\text{m}$. From the subsidence speeds presented by Kasten¹², sulphuric acid or silicate particles of radii $0.1 \mu\text{m}$ and densities 1.6 g cm^{-3} and 2.5 g cm^{-3} , respectively, injected to heights up to 50 km at the time of the eruption would have fallen to the height of the observed layer

within about 3 months and subsequently merged with the main aerosol layer below 30 km within about a year. If composed of the very low density silicates or ash also reported in larger particles near 20-km altitudes and below^{13,14}, they could remain for much longer times under subsidence. However, it is unlikely that such a thin layer could have survived more general dynamical processes; vertical turbulent diffusion, for instance, would double the width of the layer in about 1 day, even with the lowest value of coefficient currently expected ($1 \text{ m}^2 \text{ s}^{-1}$)¹⁵. If the particles represented, instead, sulphuric acid droplets produced from the oxidation of SO_2 known to be injected into the stratosphere at the time of the eruption^{16,17}, it can be shown that for an OH concentration of $\sim 3 \times 10^7 \text{ cm}^{-3}$ (ref. 18), the lifetime of the SO_2 against oxidation would have been about four days. Coagulation and growth of the sulphuric acid particles could then have occurred to produce the particles observed. However, at the temperature of 260 K existing near 40 km (deduced from the height distribution of molecular densities provided by the Rayleigh scatter measurements), evaporation should have occurred more rapidly unless the partial pressure of ambient sulphuric acid had been substantially increased. On the basis of these arguments, it seems unlikely that the observed layer could be associated with the El Chichón eruption.

If the observed layer was associated, instead, with cosmic dust particles burning out in the atmosphere, the observed thickness could have arisen from the compression of a thicker layer produced at a greater height, because of the difference in fall velocity between the top and bottom, rather than being produced *in situ*. It is found that for the heights of interest the fall velocities of particles of radii 0.003 to $10 \mu\text{m}$ as given by Kasten¹² vary with height as the inverse of the pressure. From this result the locations of initial layers of different thicknesses which could have produced the observed layer after subsidence can be calculated. For the layer observed in periods 12–14 (00.50–01.35 UT on 5 July) (Fig. 2), the half-peak points occur at 39.2 and 39.6 km. From the pressures given in the atmospheric model for midsummer at 45°N (ref. 1), it can be shown that an initial layer of thickness 5 km would have had a base at 57.3 km, almost independently of the particle radius, and thicker layers would have had slightly higher bases. However, the creation of the thin layer by subsidence implies that the particles would have a very small range of sizes. Thus for particles falling from 57.3 km to a final layer between 39.2 and 39.6 km the range of sizes can be shown to be $< 1\%$. This restriction in size need not be applicable in the case of $0.1 \mu\text{m}$ particles, if these represent the largest particles in the distribution, because smaller particles could have been distributed above the observed layer and would not have been detected by the laser radar equipment. For particles of radii $0.1 \mu\text{m}$ and densities 3.5 g cm^{-3} (ref. 19), the fall time for an initial layer of 5-km thickness with a base at 57.3 km would have been 58 days. However, the major part of this time would be spent near the final height and it is unlikely that the thin layer could have persisted for such a time in the presence of turbulent diffusion.

An upper limit for the size of the subsiding particles can be derived from the reduction in height of the maxima with time in Figs 1 and 2. The apparent downward velocity deduced is $\sim 5 \text{ cm s}^{-1}$, which for a density of 3.5 g cm^{-3} corresponds to a radius of $2.9 \mu\text{m}$ (ref. 12). The subsidence times for the layer having an initial base at 57.3 km would then be $\sim 11 \text{ h}$, and the effect of turbulent diffusion, as considered previously, would not be significant. However, in this case, the restriction on the range of sizes of the subsiding particles would apply. It would be unreasonable to associate these particles of a single size with a meteoric event.

In view of the difficulties encountered in relating the observed layer to a natural terrestrial origin or to extraterrestrial material, consideration should be given to its creation by man-made agencies. It would then be of interest to examine some of the features of this stratospheric pollution. Estimates of geostrophic wind velocities from the Stratospheric Souder Units on NOAA satellites by the Meteorological Office show predominantly east-

west winds at stratospheric heights at noon on 4 and 5 July 1983, with a mean value at the level of the cloud of $\sim 16 \text{ m s}^{-1}$. The cloud, therefore, moved from the east with that velocity and had an east-west extent of at least 170 km, based on the 3 h for which it was observed, and this implies a mass content in excess of 100 kg for each degree of latitude width.

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Displacement of the Moho by the Outer Isles thrust shown by seismic modelling

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Deep seismic data (Fig. 1) from the Caledonides, off the north-west coast of Scotland, collected (1981–82) by the British Institutions Reflection Profiling Syndicate (BIRPS), show evidence that Caledonian thrusts have been reactivated as Mesozoic normal faults^{1,2}. The best example is the Outer Isles (OI) thrust, which is mapped on land as a compressional fault, and is revealed by these data to be overlain offshore by a thick, wedge-shaped Mesozoic rift basin¹, known as the North Lewis (NL) basin. Here we have used synthetic seismic sections to identify distortions of the OI thrust reflection due to the NL basin. Models, which compensate for the effects of this distortion, show that the OI thrust has a steeper angle of dip than has been predicted previously assuming no distortion, causing the OI thrust to project into a 2–3 km offset of the Moho and hence to penetrate the entire crust. Our data, suggesting that shear strains affecting the lower crust may be localized in character, have adverse implications for rheological models which predict, to the contrary, that the lower crust is a region where strain is distributed.

Figure 2 shows the unmigrated fault reflection of the OI thrust dipping $20\text{--}22^\circ$ to the east. All angles given here were calculated using a 6 km s^{-1} velocity for converting time to depth. A second, deeper reflection from the Flannan thrust¹ dips at approximately the same angle as the OI thrust reflection. On the basis of other BIRPS data, the Flannan thrust has been interpreted to be a normal fault displacing the Moho to the west of the section shown in Fig. 2. The Flannan thrust extends upwards from within the mantle (15 s two-way travel time, or about 45 km depth) into the lower to middle crust, but does not appear to reach the surface. The OI thrust, conversely, is mapped at the surface, and can be traced on the BIRPS data to a depth of $\sim 20 \text{ km}$, or 6.8 s two-way travel time. The observation that

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reflections from both the OI thrust and the Flannan thrust apparently terminate in the lower crust led Matthews and Hirn³ to propose a model in which the two thrusts merge into a lower-crustal ductile zone, with neither penetrating the entire crust. They propose a similar model for the Himalayas, where critical-angle and wide-angle reflection data suggest the existence of Moho-displacing faults that cannot be traced to the surface⁴.

The unmigrated time section for the WINCH-1 survey (Fig. 2) shows a down-to-the-east displacement of the Moho reflection below the OI thrust, at a distance of ~33 km. The Moho reflection drops abruptly from 7.5 s two-way travel time to below 8 s. The identification of this reflection with the Moho is supported by results from a nearby refraction profile⁵. At first glance, the Moho displacement does not appear to be related to the OI thrust, as conventional time migration fails to steepen the fault plane reflection sufficiently for it to intersect the Moho at the point of displacement. Migration of the 20–22° reflection steepens it by only 1–2°. Here we use seismic modelling to show that velocity pull-down effects from the overlying NL basin mask the connection between the OI thrust and the Moho displacement; the OI thrust actually extends completely through the crust and displaces the Moho.

Velocity pull-down—the travel-time delay of reflections from structures underlying low-velocity material—can be seen on the prominent Moho reflections present in the data. On WINCH-1 (Fig. 2), these velocity effects are seen as a proportional increase in east-west travel time to the Moho as the overlying NL basin thickens. The Moho reflection is at 7.5 s two-way travel time east of the basin, and increases steadily to 8.0 s at the western edge of the data range, where the sediments are thickest. Dipping events underlying the basins, such as the OI thrust, also undergo velocity pull-down, but the distortion is not as readily apparent as the pull-down of the horizontal Moho reflection. Time migration, using a constant velocity for the overlying material, does not take this velocity-related distortion into account and will not correct for the pull-down effect.

Figure 3 shows a simple geological model for WINCH-1, developed to evaluate the geometry of the OI thrust more accurately. The model differs from the interpretations of Brewer *et al.*² and Matthews and Hirn³ in that the OI thrust dips at 29° rather than 20–25°, and extends completely through the crust to displace the Moho, rather than ending in the lower crust. Interval velocities calculated from stacking velocities obtained during original processing⁶ were smoothed and used to calculate travel times. Normal incidence, or zero-offset ray tracing, and convolution with a synthetic wavelet, produced synthetic time sections. A model in which the OI thrust dips at 29° and intersects the Moho displacement (Fig. 3) produces a synthetic seismic section (Fig. 4) which successfully matches the data. Velocity effects produced by the NL basin act to lessen the angle of the reflection from the OI thrust by 4–6° on the time section, compared with the angle it would have in a homogeneous crust. Models with velocity structures differing by up to +0.7–0.8 km s⁻¹ for the basin layers and +0.3 km s⁻¹ for the crust do not change this result significantly. However, models in which the OI thrust dips at only 20–22° do not produce a synthetic section which matches the data, unless the entire basin is composed of sediments with velocities of ~6 km s⁻¹. Sedimentary velocities of this magnitude do not match the interval velocities calculated from the data, nor do they produce the pull-down on the Moho observed in the seismic data.

The modelling results do not significantly change the geological interpretation of the Flannan thrust. However, the dip of the Flannan thrust reflection has also been decreased by velocity pull-down, and models with a dip for the Flannan thrust of 32–34° show best agreement with the original data. This thrust appears to have the same dip on the time section as the intracrustal OI thrust but, because it is most clearly seen in the upper mantle, where the seismic velocities are higher (8 as opposed to 6 km s⁻¹), a steeper dip is needed to produce a reflection parallel to the OI thrust reflection.

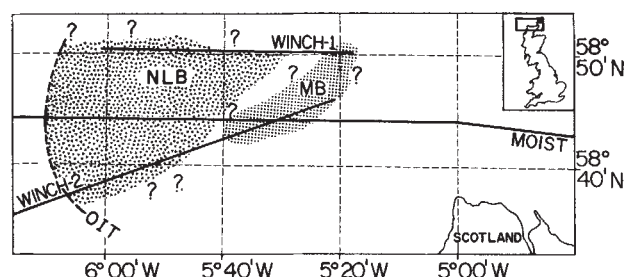


Fig. 1 Location map showing WINCH-1, MOIST and WINCH-2. Carets show the inferred extent of North Lewis basin (NLB). Note that the basin narrows to the south as it crosses WINCH-2. Dots show location of the Minch basin. The Minch basin is more fully developed on WINCH-2 and MOIST than on WINCH-1, which crosses only the shallow, northernmost portion of the basin.

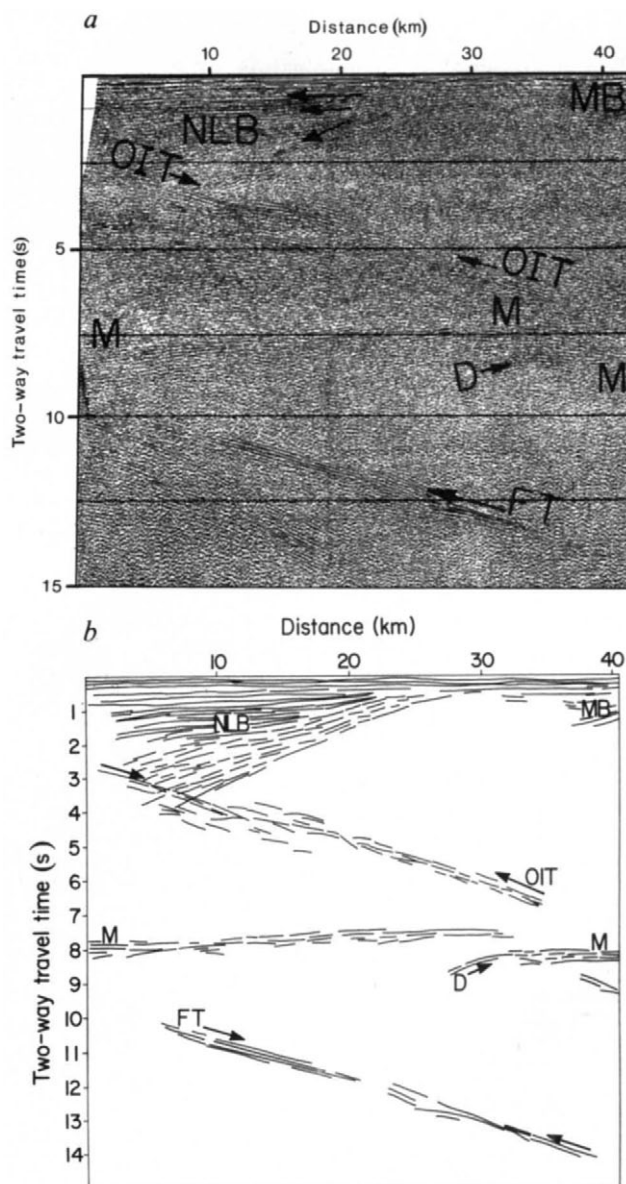


Fig. 2 a, WINCH-1, unmigrated; b, line drawing of WINCH-1. FT, Flannan thrust; OIT, Outer Isles thrust; MB, Minch basin; NLB, North Lewis basin; M, Moho. Note a 600–700 ms down-to-the-east displacement of the Moho at 33 km. The OI thrust reflection dips at 20–22°. The shallow, overlying Minch basin contributes slightly to the displacement of the Moho by adding a negligible amount of velocity pull-down. The Minch basin contributes ~500 ms of pull-down on the Moho seen on MOIST and WINCH-2, where the basin is much better developed. Event D fits diffraction curves for crustal velocities. The presence of a diffraction suggests that the displacement is real rather than a velocity effect.

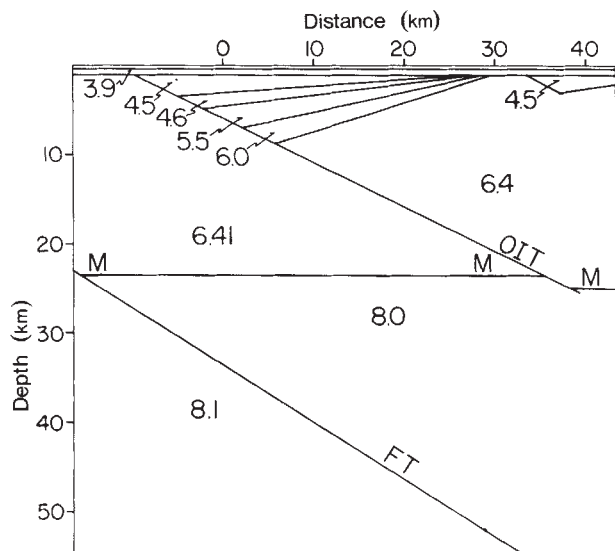


Fig. 3 Geological model for the OI thrust in which the fault actually dips 29° and displaces the Moho at 33 km. The Flannan thrust dips at 33°. Velocities were smoothed from interval velocities obtained by BIRPS during processing of the data⁶, and are in km s⁻¹. Although these velocities are relatively high, the sediments comprising the NL basin are believed to be mainly Palaeozoic (D. K. Smythe, personal communication).

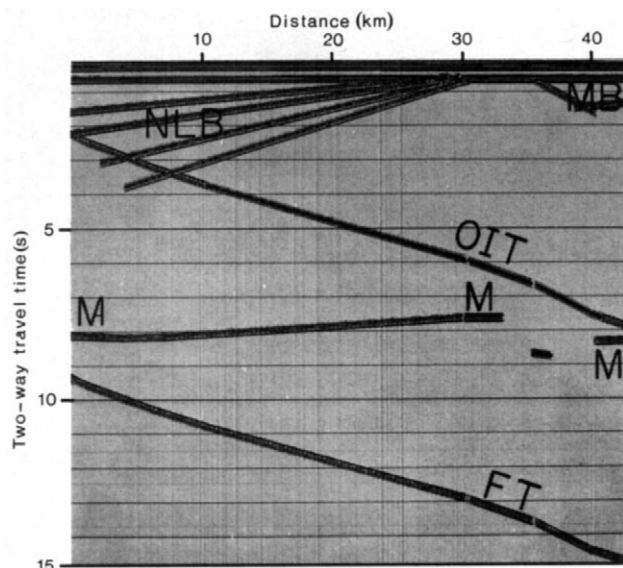


Fig. 4 Synthetic seismic section for the model in Fig. 2. Although the OI and Flannan fault planes dip at 29° and 33°, respectively, their reflections dip only 20–22° on the seismic section. If the NL basin did not overlie the OI thrust, the reflection from the fault would dip at 26° on the unmigrated seismic section.

Three-dimensional control of these structures is provided by MOIST, a previous BIRPS survey¹, parallel to WINCH-1, but extending much further east, and by WINCH-2, which intersects MOIST in the Minch basin (Fig. 1). The OI thrust reflection dips at 20–22° on MOIST and 24–25° on WINCH-2. The observation that the NL basin is less well developed on WINCH-2 agrees well with its steeper dip on this line. All three seismic lines are as close as possible to dip lines, based on unpublished shallow data (D. K. Smythe, personal communication). Similar modelling suggests that a Moho displacement is associated with the OI thrust on both MOIST and WINCH-2. However, the displacement is obscured on MOIST and WINCH-2 by velocity pull-down associated with the Minch basin, which is much thicker on these lines than on WINCH-1. The OI and Flannan thrusts are most successfully modelled on MOIST by dips of

29° and 32–33°, respectively, as for the parallel WINCH-1.

The OI thrust reflection ends at a much shallower depth on MOIST than on WINCH-1, where it can be traced almost completely through the crust. The termination of the OI thrust reflection corresponds in each case to the beginning of the Minch basin at the surface, suggesting that the absence of a reflection from the OI thrust in the lower crust is owing to complications associated with the Minch basin sediments, rather than the termination of the fault at different crustal levels on the two lines.

Brewer and Smythe^{7,8} have proposed that the OI thrust displaces the Moho on MOIST, based on the intersection of the Moho with an extrapolation of the migrated OI thrust fault-plane reflection. However, because the apparent dip of the migrated fault reflection (22–23°) differs from the dip of the fault plane by 6–7°, through pull-down, these extrapolations lead to an intersection ~20 km east of the displacement identified here. The apparent Moho displacements seen on MOIST at these other locations are caused by velocity pull-down from overlying basins; it is simply coincidental that the distorted OI thrust reflection projects into one of these apparent displacements.

In conclusion, the new modelling indicates that the OI thrust does not end in a ductile zone in the lower crust, but continues completely through the crust into the uppermost mantle. Although this result does not preclude the existence of a ductile lower crust, it does demonstrate that localized zones of deformation are likely to exist at these depths.

The synthetic seismic sections presented here were calculated using the Advanced Interpretive Modelling System (AIMS, Gequest International) on the Megaseis (Seiscom Delta) seismic computing system operated by the Consortium for Continental Reflection Profiling at Cornell. The research was supported by NSF grants EAR82-12445 and EAR83-13378. These results were presented at the International Symposium on the Deep Structure of the Continental Crust at Cornell University, 26–28 June 1984. I thank Simon Klemperer, David Smythe, Larry Brown and Michael Warner for comments. Contribution 805 of Department of Geological Sciences, Cornell University.

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Palaeoclimate of Baffin Bay from 300,000-year record of foraminifera, dinoflagellates and pollen

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Several different models^{1–5} have attempted to relate Quaternary marine and terrestrial records of ice-sheet growth in the North Atlantic region. The models are derived from either discontinuous terrestrial records, including marine fossils in raised shorelines^{1,2}, or from deep-sea calcareous microfossils^{3,4}. This study correlates Arctic marine and terrestrial data directly by comparing $\delta^{18}\text{O}$, foraminiferal, dinoflagellate and pollen records from a core taken in Baffin Bay. The core contains an almost continuous sequence of sediments deposited during isotope stages 1–10. Subarctic foraminifera and Atlantic dinoflagellates in early isotope stages

2, 4, 6 and 8 show that subarctic water entered southern Baffin Bay before the glacial maxima. Boreal-subarctic pollen suggest that Atlantic air flowed into Baffin Bay during the ice sheet growth phases. These data support models which postulate that open water in the Labrador Sea had an important role in supplying moisture to Laurentide and Greenland ice sheets.

Data for this study of ocean-atmosphere interaction during the late Quaternary were obtained from core 77-027-017 taken from a water depth of 935 m on the northern slope of Davis Strait at 66° 54.1'N, 58° 17.7' W (Fig. 1). The core includes a thin surface layer of brown diatomaceous mud overlying detrital carbonate-rich gravelly sand and ~10 m of partially bioturbated olive-grey sandy or silty mud. Planktonic and benthic foraminifera were studied in the >63 µm fraction using standard procedures⁶ to provide a biostratigraphical and palaeoceanographic record. Previous studies in Baffin Bay⁷ indicate that calcium carbonate dissolution limits the information content of calcareous microfossils; therefore, pollen and dinoflagellate cysts were also studied in core 017, using extraction methods⁸ suitable for clayey muds. Pollen and spores in marine sediments provide a link between terrestrial and oceanic environments and, off eastern Canada, pollen assemblages show a strong correlation with onshore climatic zones⁸. Most dinoflagellate cysts are spores of planktonic algae and, in marine sediments, they are proxy-indicators of surface water conditions^{9,10}.

The oxygen isotope composition of planktonic foraminifera in core 017 (Fig. 2) shows a typical isotopic record extending into stages 10 as defined by Emiliani¹¹. Calcareous foraminifera are absent from the top of the core because of the present shallow lysocline⁷. Gastropod shells from ~50 cm depth yielded a ¹⁴C age of 10,530 yr BP, confirming the isotope stage 1-2 transition between 60 and 70 cm. From 75 to 300 cm, high $\delta^{18}\text{O}$ values represent isotope stages 2, 3 and 4. At 300 cm, a large (2.5%) shift in $\delta^{18}\text{O}$ marks the top of stage 5. Stage 6 (465–515 cm) is rather compressed. Stage 7 (515–630 cm) has several light isotopic peaks, and a thermoluminescence (TL) age of $281,000 \pm 20\%$ yr BP on silt from 625 to 627.5 cm generally agrees with the age¹¹ of 251,000 yr BP for the base of stage 7. Stages 8 and 9 contain two light peaks. Below 810 cm, the record lies within stage 10.

Although the number and diversity of planktonic and benthic foraminifera in core 017 (Fig. 2) are low, there are cyclical variations in their percentage abundance. Stage 1 contains only arenaceous benthic foraminifera, typically *Textularia earlandi*, *Trochammina inflata*, *T. nana*, *Eggerella advena* and *Reophax arctica*, with abundant diatoms and radiolaria. In the Canadian Arctic, this benthic assemblage is generally confined to areas of cool saline bottom waters¹², and is often associated with carbonate dissolution⁶. Benthic foraminifera in stages 3, 5, 7 and 9 are mostly calcareous species dominated by *Cassidulina reniforme*, *Elphidium excavatum* and *Fursenkoina fusiformis*. This fauna characterizes silty sediments below cold and low-oxygen waters. Planktonics are almost all *Neogloboquadrina pachyderma* (sinistral) with abundant diatoms and radiolaria. Calcareous foraminifera in stages 3, 5, 7 and 9 are largely lost to dissolution⁷. Benthics in stages 2, 4, 6, 8 and 10 are predominantly calcareous, mainly *Islandiella teretis*, *Nonionella labradorica*, *Buccella frigida*, *Astronion gallowayi* and *E. excavatum*. This fauna presently occupies deep basins with relatively warm bottom water on the eastern Canadian margin¹².

Planktonic foraminifera in core 017 include *N. pachyderma* (sinistral and dextral), *Globigerina quinqueloba* and *G. bulloides*, with up to 15% subarctic species. Modern distributions of subarctic planktonics are confined to warmer (4–10°C) surface waters; beneath the West Greenland Current, *G. quinqueloba* and *G. bulloides* make up to 25% of the planktonic fauna. The presence of subarctic foraminiferal peaks in the early glacial stages of core 017 suggests significant advection of subarctic surface water into northern Labrador Sea at these times (refs 6, 13, and manuscript in preparation). This would occur if the Arctic Island channels were largely blocked by ice, thereby reducing Arctic water outflow to the North Atlantic. Decreased

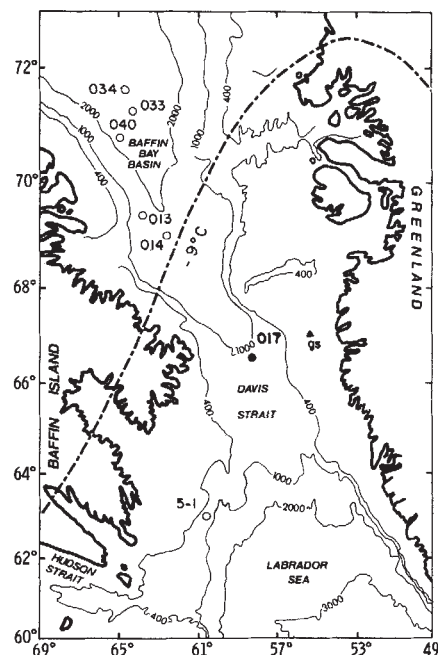


Fig. 1 Bathymetry of Baffin Bay (contours in m) showing the location of core 017 and other cores (ref. 13 and work in preparation) with detailed oxygen isotope, microfossil and/or palynological data; gs, grab sample used for isotopic data at the top of core 017 (see Fig. 2). The -9°C mean annual air isotherm (---) marks the average position of the Atlantic-Arctic air front.

surface runoff and reduced north-east Atlantic inflow would also result in a weaker Baffin Land Current during glacial periods⁶, thus allowing northward penetration of subarctic waters.

Data from core 017 and other cores from Davis Strait^{6,13} show that the interglacial strata are depleted in planktonic foraminifera but rich in siliceous/arenaceous microfossils, whereas the opposite is true for glacial strata. Alternation of predominantly siliceous/arenaceous and calcareous microfossils is typical of areas where carbonate cycles are a function of dissolution cycles⁷. Therefore, acid-resistant palynomorphs were studied in 42 samples taken as closely as possible to the foraminiferal sample intervals.

Figure 3 shows that Quaternary dinoflagellates are common although the numbers generally decrease downcore. The Atlantic dinoflagellates are species which presently have maximum percentage abundances in the temperate North Atlantic¹⁰, particularly beneath the North Atlantic Drift. These species include *Operculodinium centrocarpum*, *Nematosphaeropsis labyrinthea*, *Spiniferites ramosus*, *S. elongatus*, *S. bulloides* and *S. membranaceus*. Their modern distribution in Baffin Bay⁹ is confined to deep-water areas beneath the relatively warm (2–5°C) West Greenland Current. In Core 017, these species occur mainly in the interglacial stages 1, 5 and 7, but they also occur in glacial isotopic stage 2 and in the lower parts of stages 4, 6 and 8. The most common species in core 017 is *Multispinula minuta* which dominates the Canadian Arctic dinocyst assemblage⁹ found in modern sediments below the cold Baffin Land and Labrador Currents. In core 017, however, *M. minuta* dominates only in the glacial stages 6, 10 and upper 8, where it is associated with *Brigantidium simplex*, *Peridinium faeroense* cysts and a *Leiosphaera* species⁹ which is presently abundant in Arctic channels north of Baffin Bay.

The main features of the dinoflagellate stratigraphy are (1) peaks of Atlantic species which often correspond to decreases in subarctic foraminifera; (2) changes in the ratio of the warm water indicator *O. centrocarpum* to the Arctic indicator *M. minuta*, with peaks of *O. centrocarpum* often inversely related to the subarctic foraminiferal peaks; and (3) large increases in dinoflagellate concentrations which mostly correspond to

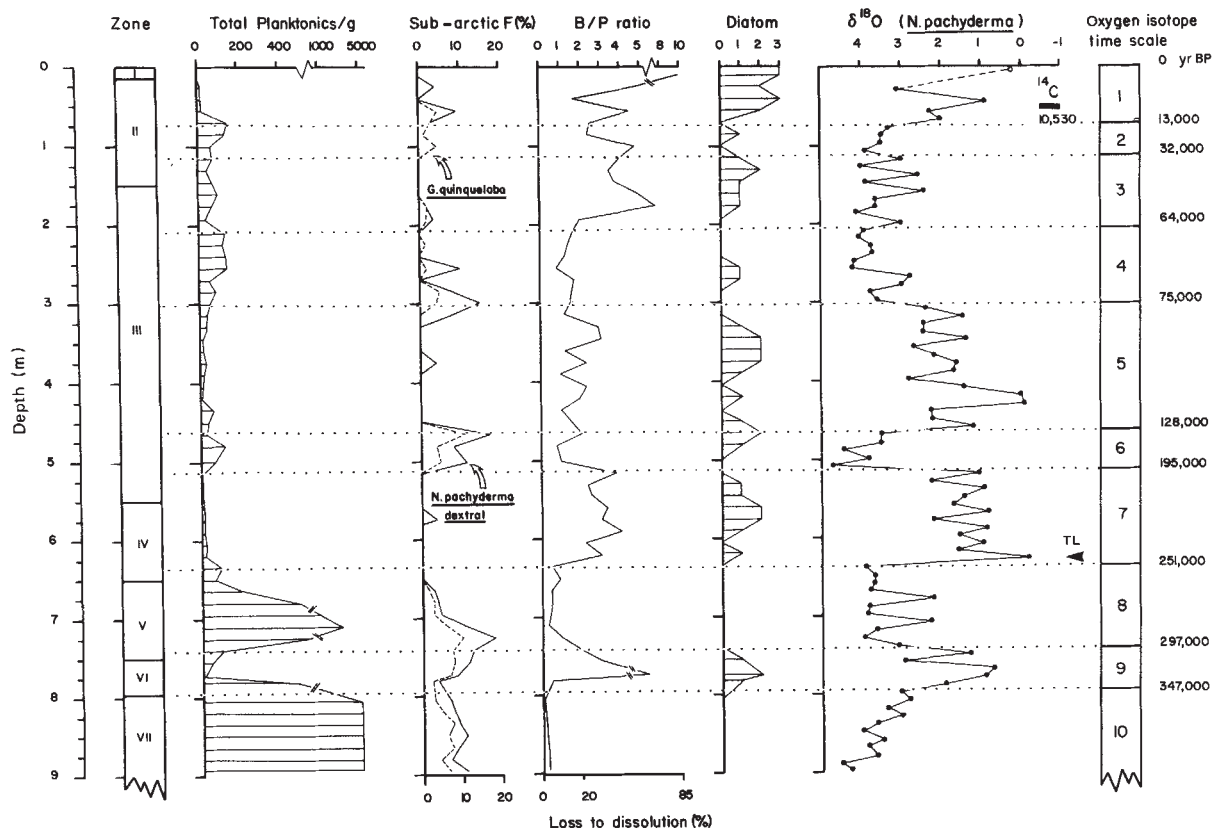


Fig. 2 Foraminiferal zones, planktonic foraminiferal totals per gram sediment, subarctic foraminiferal species (percentage total planktonics), large diatoms ($>63\ \mu\text{m}$) per gram sediment on a scale from 0 (absent) to 3 (abundant, $>10,000$), and $\delta^{18}\text{O}$ record of *Neogloboquadrina pachyderma* (sinistral) in core 017. Oxygen isotope time scale refers to ref. 11. ^{14}C , radiocarbon data; TL, thermoluminescence data; B/P ratio is the ratio of total benthic to total planktonic foraminifera. Dashed line at top of the isotope record was obtained from the grab sample shown in Fig. 1.

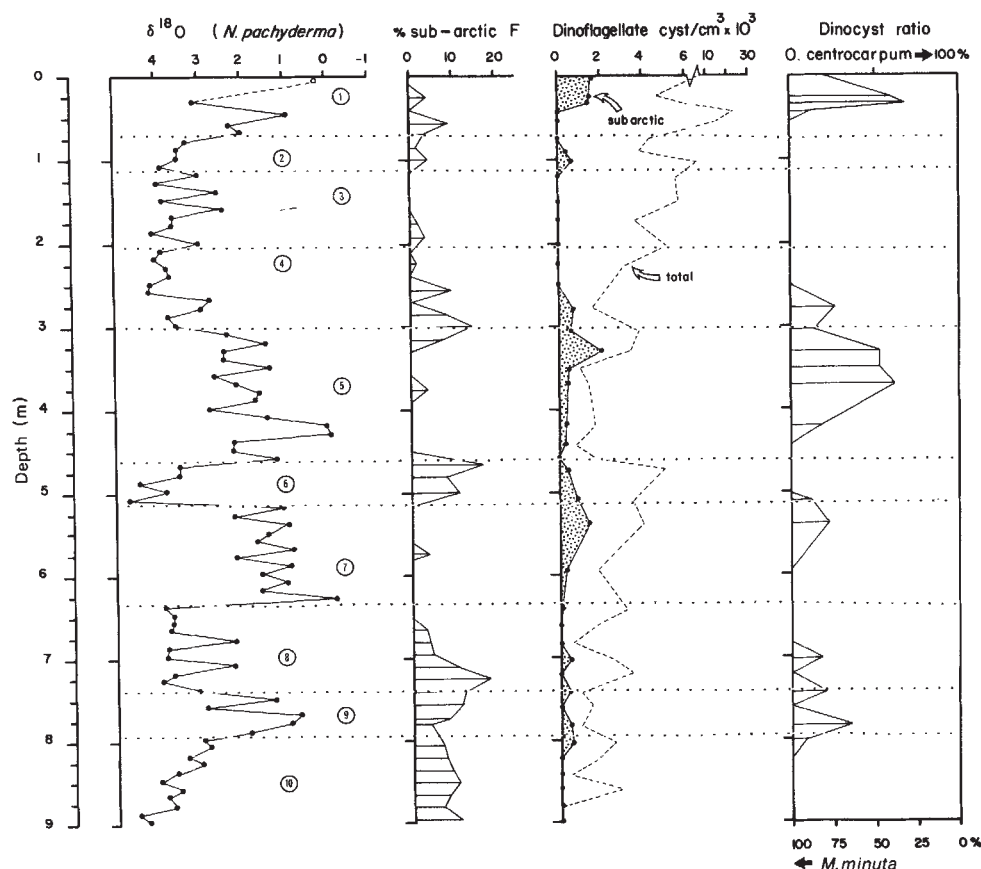


Fig. 3 Summary of dinoflagellate cysts in core 017, compared with the $\delta^{18}\text{O}$ record and percentage total sub-arctic planktonic foraminifera. See text for explanation of subarctic (= Atlantic) dinoflagellates and the Dinocyst ratio. Circled numbers refer to isotopic stages.

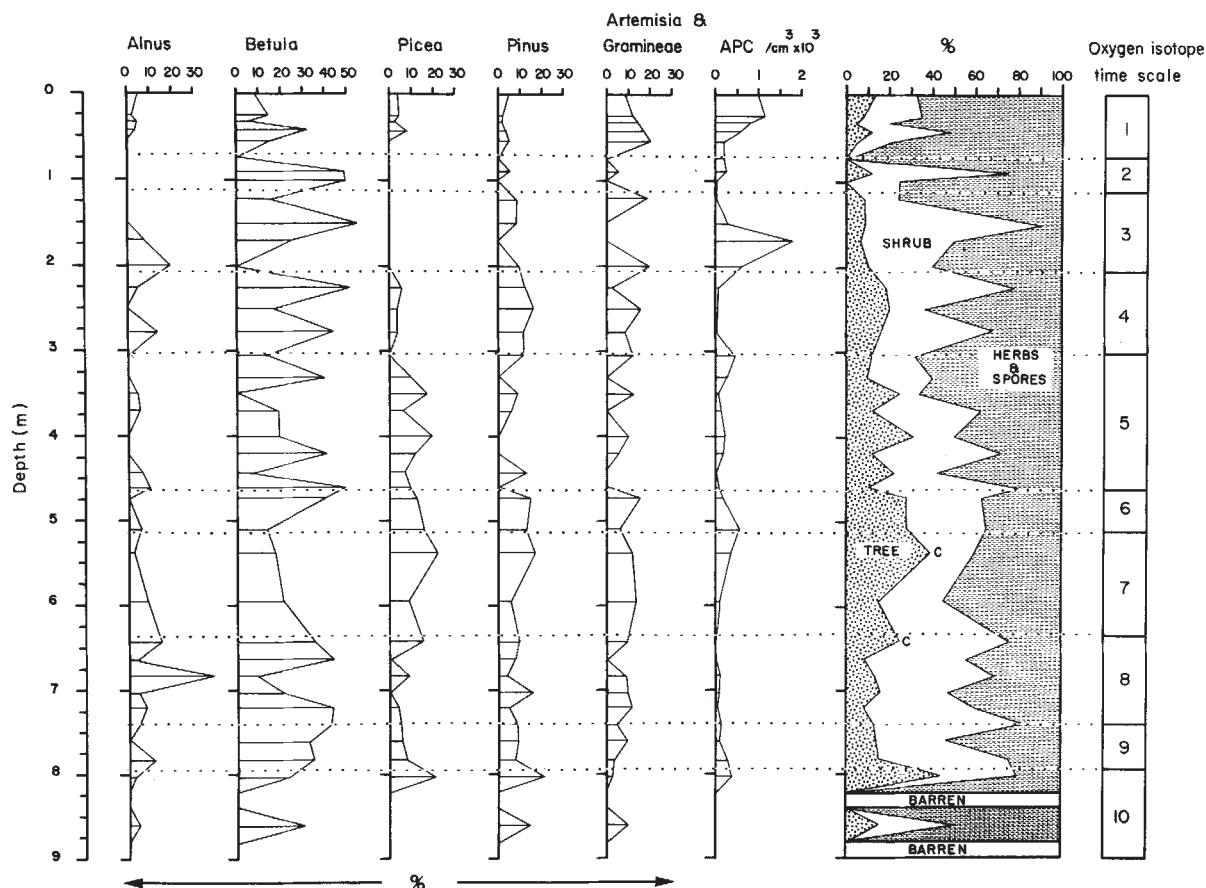


Fig. 4 Pollen percentage diagram for main tree, shrub and herb taxa in core 017, total concentrations of Quaternary pollen and spores (APC) and cumulative percentages of conifer tree, 'shrub' (*Alnus* and *Betula*) and herb pollen plus moss spores. C, *Carya* pollen present.

interglacial stages and are correlated with diatom peaks. These features indicate that maximum primary productivity during the late Quaternary is associated with interglacial stages of relatively warm, open summer water in southern Baffin Bay. The productivity peaks may account for the greater dissolution (20%) of planktonic foraminifera during interglacials than during glacial stages (<10% dissolution)⁷. However, the presence of Atlantic dinoflagellates in stages 2 and lower stages 4, 6 and 8 also implies relatively warm surface water at the core site during early parts of glacial stages, providing independent confirmation of the palaeotemperatures suggested by the subarctic foraminifera.

As for the foraminifera, the number and diversity of quaternary pollen and spores in core 017 (Fig. 4) are low, as expected for oceanic sediments north of the treeline in eastern Canada^{8,9}, but again there are cyclical variations in 'absolute' pollen concentrations (APC) and percentage abundance of indicator species which are correlated with oxygen isotope stages. The top of stage 1 (0–50 cm) is characterized by boreal-subarctic shrub (*Alnus*) and tree pollen percentages (*Pinus*, *Picea*) similar to those found in surface sediments of Baffin Bay⁹, south of the summer position of the Atlantic–Arctic air front (Fig. 1). About 75% of the *Betula* pollen are the small *B. minor* type typical of tundra shrubs. Gramineae dominate the herb pollen, with other taxa including *Artemisia*, *Oxyria*, Rosaceae and Saxifragaceae. The interval from 50 to 325 cm is marked by rare *Picea* and by large fluctuations in *Betula*. Stage 2 contains low APC and variable percentage *Pinus*, Gramineae, *Artemisia*, *Sphagnum* and *Polytrichum*, as presently found in marine sediments⁹ north of the Atlantic–Arctic air front. *Betula* percentages are similar to those found in the Devon Island ice core for the same time interval¹⁴. Stage 3 is marked by higher APC and *Alnus* occurs at the base, suggesting some influx of southern air¹⁵. Stages 4,

8 and 10 contain very low APC but *Alnus* and *Picea* suggest sporadic influx of Atlantic air. Stage 5 is marked by higher tree pollen percentages and peaks in *Picea* and *Alnus*; the assemblages are similar to those in stage 1, but higher percentage *Betula* suggests a wider shrub distribution than presently found on Baffin Island. This agrees with pollen in peat below the Kogalu marine sediments¹⁶, with an age of ~130,000 yr BP.

Tree pollen in stage 6 suggest a strong influx of Atlantic air, but sampling control is poor for this interval in core 017, and data from other cores (Fig. 1) show that this is true only for sediments near the stage 6–7 transition. Interglacial stages 7 and 9 show slight increases in APC and percentage tree pollen; the presence of *Carya* also marks stage 7 in core 017 and other Davis Strait cores¹³.

Overall, the combined palynological and foraminiferal data from core 017 provide palaeoclimatic evidence which supports models^{1–3} that attribute North Atlantic continental ice sheet growth to increased northward flow of warm water during early glacial stages. The present relatively warm climate and sea-surface temperatures in southern Baffin Bay result from (1) high latitude penetration of warm cyclonic air fronts; (2) inflow of Atlantic water through the West Greenland Current. Meridional air flow accounts for boreal tree and shrub pollen in modern Baffin Bay sediments^{9,15} and during older interglacial stages. Lower pollen concentrations and diversity during glacial stages suggest a southward shift of the Arctic air front and stronger zonal flow of polar air characterized by *Betula*, *Artemisia*, Gramineae and other herbs, in addition to ubiquitous *Pinus* grains. However, the presence of boreal tree/shrub taxa in glacial stages 4, 6, parts of 8 and 10 indicate influx of Atlantic air during all initial and some later phases of ice-sheet growth. Presence of Atlantic dinoflagellates during early glacial stages also

supports the foraminiferal data indicating increased water mass transport from the south. Furthermore, the persistent presence of pollen and dinoflagellates during glacial maxima implies extensive areas of summer open water (for example, more than the present ice covered Arctic Ocean north of Ellesmere Island¹⁷), although production of dinoflagellates and large (>63 µm) diatoms is low. Heterotrophic peridinioid dinoflagellates like *M. minuta* (= *Protoperidinium pellucidum* cyst form) can survive under ice up to 2 m thick, but they are very rare or absent in continuously ice covered regions¹⁷.

We conclude that both palynological and foraminiferal studies of core 017 provide palaeoclimatic data which contradict models requiring continuous sea-ice cover in Baffin Bay/Labrador Sea during glacial stages⁵ but are compatible with models based on orbital changes in summer insolation, with very rapid initial glaciation¹⁻³ and subsequent limited sea ice, the continental ice sheets being maintained by precipitation transported northwards from the south-west along storm tracks directed by strong land-sea thermal gradients.

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Inheritance of stereotyped gibbon calls

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Little is known about how vocal patterns develop in non-human primates, mainly because suitable controlled experiments are difficult to carry out on these animals¹. Results of isolation experiments²⁻⁴ and observations of interspecific hybrids^{1,5} suggest no greater role for vocal learning than exists in many other vertebrates⁶⁻⁹, and less than has been found in birds¹⁰⁻¹³. We have now studied vocal patterns of hybrids between white-handed gibbons (*Hylobates lar*) and pileated gibbons (*Hylobates pileatus*) in natural mixed-species groups, in a zone of interspecies contact in central Thailand, and in some captive mixed-species groups. We find that in female hybrids, the patterns of the loud and stereotyped 'great-calls' show no evidence of learning from parents, and appear to be under strong genetic control. Daughters maturing in groups with genetically unlike parents develop great-calls unlike those of their mothers, even though these calls develop only while the daughters sing simultaneously with their mothers.

Great-calls are the most spectacular sounds in the repertoires of gibbons. They are sung by females at intervals of 2-4 min

during morning song bouts, and last approximately 14-24 s. In most species, females are accompanied by their mates in a ritualized duet¹⁴⁻²⁰; in a few species, females sing solo near their mates^{16,20-22}. The songs are believed to function in territory advertisement^{5,14,16,23} and in maintaining the monogamous pair bond^{15,19,24-26}.

The acoustic patterns of great-calls are highly species-specific^{16,27,28}. The great-calls of *H. lar* and *H. pileatus* have similar length and frequency range, the major differences being in the shapes and number of notes (Fig. 1). Great-calls of *lar* average 8.4 notes (s.d. 1.66; $n = 14$ individuals recorded in Khao Yai Park, Thailand, one call randomly selected from each) and *pileatus* great-calls average 72.8 notes (s.d. 9.61; $n = 19$ individuals, recorded from three locations in Thailand)²⁹. Comparison of sound spectrograms of *lar*, *pileatus* and hybrid calls shows that the number of notes per s uttered at the end of the call varies relatively less than the total number of notes. For *lar*, the number of notes per s in the last quarter of the call (including no less than three notes) averages 0.49 (s.d. 0.054; $n = 14$) compared with an average of 9.6 (s.d. 0.51; $n = 19$) for *pileatus*²⁹.

Most of our recordings of wild gibbons were made in a small zone of overlap and hybridization between *H. lar* and *H. pileatus* in Khao Yai National Park, central Thailand^{27,29,30}. About 215 groups were identified and mapped in this zone during 1976-80, and 23 groups were found to contain mated adults of different species or hybrids, most of which have been tape recorded. All tape-recorded hybrids have been frequently studied closely with binoculars.

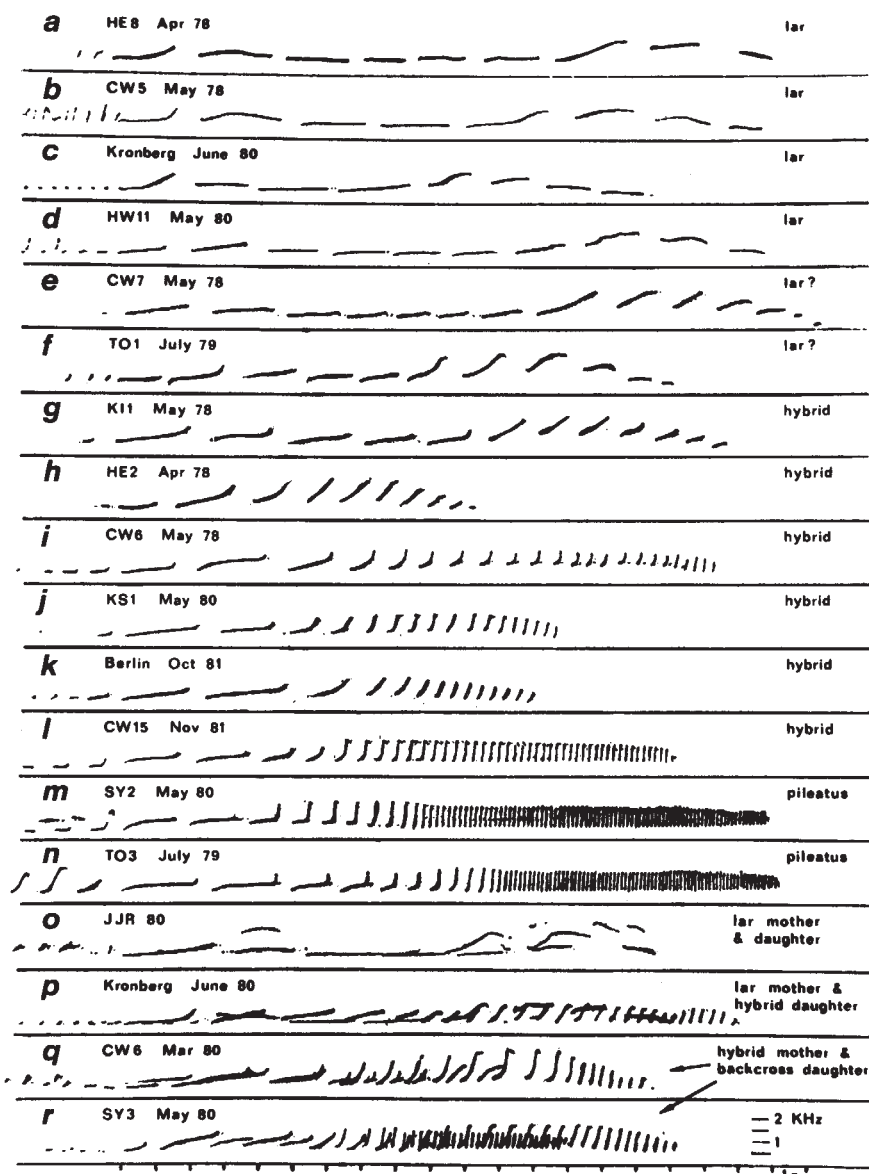
Despite their close relationship, body colour patterns provide unambiguous separation of the species and recognition of their hybrids²⁹. The major pelage differences are the presence of a complete ring of white fur around the face in *lar*, compared with white eyebrows and a black cap in *pileatus*; also a white scrotal tuft of fur in *pileatus* males, and a black 'vest' and greyish body in *pileatus* females, compared with the solid buff or brownish-black body coloration in *lar*. *H. pileatus* is sexually dichromatic whereas *lar* is asexually dichromatic in Khao Yai. Hybrids have various odd or intermediate colour patterns. No individuals with normal *lar* or *pileatus* call types have been found to have odd or atypical coloration, but a few individuals with *lar*-like coloration (for example, females CW7 and TO1, Fig. 1) have slightly more rapid emission of notes than is typical of *lar*.

Great-call patterns of clear phenotypic hybrids tend to be intermediate between those of *lar* and *pileatus* in nearly all respects. Those hybrids that resemble one species more than the other (Fig. 1g, h, i)²⁹ show a corresponding resemblance in great-call pattern. One feature that is somewhat more variable in hybrids than in *lar* or *pileatus* is the length of the call; some have fairly short calls (for example, HE2). In respects other than acoustic pattern, such as frequency and time of singing, duet structure and loudness, the hybrids have not been found to differ significantly from normal *lar* or *pileatus*, although in some respects they are intermediate between the species (our unpublished data).

The relatively rigid territoriality and monogamy of gibbons³¹ allows the identification of mother-daughter pairs in the wild with a high degree of certainty. Juvenile or sub-adult females in the family group frequently sing along with their mother's great-calls, the mother and daughter usually beginning their calls within 1 s of each other^{15,18,22,32-34}. This suggests that the young may have to learn or practise the song³⁵, which does not seem to serve any important function before adulthood.

Three groups with genetically unlike parents (as determined by pelage) had 4-5-year-old daughters who sang with their mothers. The pelage of the daughters was intermediate between that of parental types, providing further evidence of parentage²⁹. Sonagrams of two of these pairs of females are shown (Fig. 1q, r). In all three cases the adult females were hybrids, with call patterns and pelage about half-way between *lar* and *pileatus*. In two cases (CW6 and HS3), the adult male was a *lar*; in the

Fig. 1 Sound spectrograms of representative great-calls of female *H. lar*, *H. pileatus* and hybrids. The great-calls begin near the first 1-s mark on the scale at the bottom; the first 3 s of the sonagrams consist of introductory 'hoos' by the female and (cases *b*, *d*, *o*, *q*) notes by the male partner. When more than one call of a female was available on tape, the one chosen for spectrography was selected randomly. All calls except *c*, *k* and *p* were recorded in Khao Yai Park, Thailand. Great-calls were recorded from four *lar* females (*a*–*d*), two *lar*-like females, possibly backcrosses, with slightly more notes than normal (*e*, *f*), hybrids with odd but somewhat *lar*-like pelage (*g*, *h*), two hybrids with intermediate pelage (*i*, *j*), an F₁ hybrid in the Berlin Zoo (*k*), a wild hybrid with odd but *pileatus*-like pelage (*l*), two *pileatus* females (*m*, *n*), mother and daughter *lar* (*o*), *H. lar* mother and daughter with *pileatus* father (*p*), hybrid mother and backcross daughter with *lar* father (daughter's call is shorter and ends with more horizontal notes) (*q*), hybrid mother and backcross daughter with *pileatus* father (daughter's call is faster) (*r*). Tape recordings in the field were made with a Nagra SNN recorder with Sennheiser MKH 815 microphone; those of the zoo animals were made with a Uher 4000 Report S recorder with M534 microphone. Sound spectrograms were made on a UA-Ubiquitous spectrum analyser (Nicolet Corporation).



third (SY3), he was a *pileatus*. The daughters' calls were a few seconds shorter than the mothers' and not as loud, as is typical of the calls of juveniles.

Group CW6 was first seen and recorded in May 1978, and was again recorded as a trio in March 1980. The daughter's call still differed from her mother's in the slower rate of emission of notes. By 1981, the call had lengthened slightly and become louder, but the pattern remained the same. In the second group with a hybrid adult female and a *lar* adult male, the juvenile female sang a great-call similar to that of the CW6 juvenile. In group SY3, with a *pileatus* adult male, the daughter's great-call was more rapid than that of her mother, and resembled that of female CW15, a *pileatus*-like adult which, judging from pelage, is probably also a backcross to *pileatus*²⁹ (Fig. 1*r*, *l*).

In all of these mixed groups, the hybrid adult females sang vigorously and at least as frequently as normal *lar* and *pileatus* females. The young have therefore heard a daily average of roughly 8–10 great-calls sung by their mothers throughout their lives³¹.

The songs of two zoo hybrids have also been recorded and sonagraphed (Fig. 1*k*, *p*). One is an offspring of a cross between a light-phase *lar* female and a *pileatus* male, in the Kronberg Zoo, West Germany. The hybrid was 10 years old when recorded and was kept with her parents and two brothers in a large

enclosure where the parents and daughter sang regularly as a trio. The daughter had a great-call of somewhat variable length but usually with 20–26 notes, and with a frequency of notes reaching 2.9 s^{-1} , similar in pattern to some recorded in Khao Yai Park. The other hybrid, born in the Zurich Zoo in 1970 but now located in the Berlin Zoo, is the offspring of a dark-phase *lar* female from Thailand and a *pileatus* male. Its great-call usually had around 15–20 notes, and attained a rate of 2.6 notes per s.

The rate of emission of notes in the great-calls of the zoo F₁ hybrids lies approximately half-way between that of the parental species on a logarithmic scale (Fig. 2). Likewise, the wild hybrids with similar call types (CW6, HS3 and SY3 mothers) have backcross daughters with great-calls which regress half-way (or slightly more) to their fathers' species on this scale. Although it is difficult to postulate the unlearned pattern of a hybrid's call, if learning were present we should expect greater similarity to the mother's call in backcrosses either to *lar* or to *pileatus*, or both. No backcross, however, shows greater resemblance to the mother's genetic type. Although the backcrosses available are still few in number, the data are consistent with the hypothesis that genes for great-call pattern have multiplicative effects on the rate of emission of notes and are inherited in a quantitative fashion.

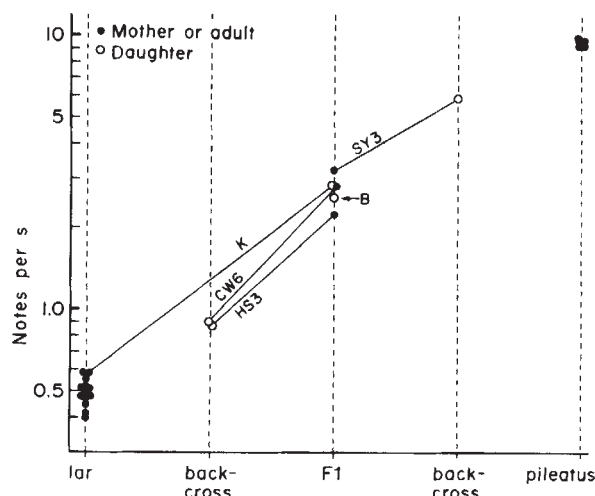


Fig. 2 Number of notes per s in the last quarter of the great-call (including no less than three notes and intervals), on a logarithmic scale in relation to genetic parentage, for *H. lar*, *H. pileatus* and hybrids. The calls of the CW6, HS3 and SY3 adults have been placed as though they were F₁ hybrids, although their parents were not known. The CW6 and HW3 daughters are backcrosses to *lar*; the SY3 daughter is a backcross to *pileatus*. The 12 *lar* and 5 *pileatus* calls were recorded in Khao Yai Park, Thailand, each from a different animal. Mother-daughter pairs are connected by lines. B, Berlin; K, Kronberg.

Intermediate call patterns in hybrids indicates that they could not have strictly learned the patterns from their mothers; had they done so either the *lar* or the *pileatus* call type would have been passed through the generations. As adult males do not give calls resembling great-calls, it is difficult to imagine how the young could have learned great-call patterns from parents at all. The possibility still exists that the young could have been influenced by females in neighbouring groups. This can be ruled out only in the case of the Kronberg female. The female, born in the Zurich Zoo, heard great-calls of her hybrid older sister before she was 2 years old (C. Schmidt, personal communication). Her sister (born in 1961) was also reported to give calls unlike those of her mother, but recordings are not available. No female *pileatus* was in the zoo at this time. As for the wild groups, CW6 was surrounded by *lar* groups, including CW7 (Fig. 1), from 1976–80. The HS3 daughter heard both *lar* and *pileatus* great-calls, and some in the distance that sounded like hybrids. Group SY3 was surrounded by *pileatus* groups, with one or two *lar* groups audible in the distance, but none that sounded exactly like the backcross daughter.

Thus, although singing with parents may be important to the timely development or maturation of the great-call in young females, or for learning its proper timing and context, the acoustic pattern of the final result seems to be resistant to alteration by learning in the natural social setting, and under strong genetic control.

In some respects, great-calls are not typical gibbon vocalizations. No other phrases of the repertoire are as long and stereotyped, although great-calls do display individual variability³⁶. The lack of modification by learning may therefore not be true of all vocal patterns of gibbons. Conceivably, the rigidity of great-call patterns is related to the hypothesized role of duets in strengthening the pair bond, in providing a key stimulus which reinforces the male's fidelity to mate and territory.

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Inositol 1,4,5-trisphosphate microinjection activates sea urchin eggs

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When a sperm activates a sea urchin egg at fertilization, it triggers the release of calcium from a store within the egg by a hitherto unknown mechanism^{1,2}. The ensuing transient increase in cytoplasmic calcium concentration is the stimulus for the beginning of embryonic development^{3,4}. Here we have activated sea urchin (*Lytechinus pictus*) eggs by microinjecting inositol 1,4,5-trisphosphate (InsP₃), a substance which Berridge⁵ has suggested may be an intracellular messenger whose probable function is to release calcium from intracellular stores^{6–9}. It is thought that InsP₃ is produced from plasma membrane phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) via enzymatic hydrolysis by a phospholipase C¹⁰. Recently, it has been demonstrated that there is a substantial increase in the turnover of polyphosphoinositides in the sea urchin egg at fertilization¹¹. We now present preliminary

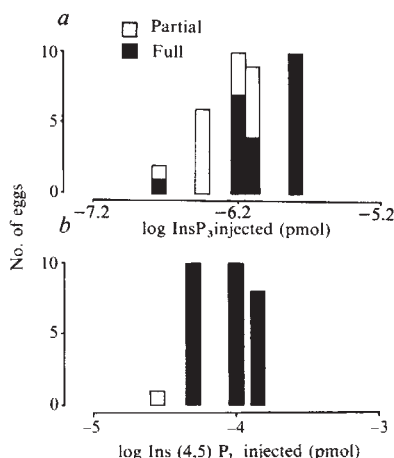


Fig. 1 Elevation of the fertilization envelope induced by microinjection of inositol phosphates: *a*, InsP₃; *b*, Ins(4,5)P₂. The pipette tip was placed eccentrically within the egg. Partial fertilization envelopes arose close to the tip of the pipette. Each amount of InsP₃ indicated was tested by microinjecting 10 eggs; these amounts correspond to 4, 8 or 20 pl of 100 nM, 4 pl of 400 nM or 4 pl of 1,000 nM InsP₃. 8 pl of 20 nM (1.6×10^{-7} pmol) InsP₃ was ineffective. 115 eggs injected with amounts of InsP₃ ranging from 5×10^{-6} to 3×10^{-4} pmol (injected volume 0.5 pl) all showed complete fertilization membranes. 2×10^{-5} pmol Ins(4,5)P₂ was ineffective. The amounts correspond to 1, 4 or 5 pl of 25 μ M or 0.5 pl of 100 μ M Ins(4,5)P₂. Volumes injected were estimated by measuring the diameter of droplets extruded under paraffin oil. Inositol phosphates were prepared as described by Irvine *et al.*²¹; each compound was dissolved in 0.5 M KCl containing 100 μ M EGTA, pH 7.0, and microinjected through bevelled micropipettes using pressure pulses from a nitrogen cylinder⁴. Microinjection of vehicle alone caused activation in 1 of 50 eggs. Free calcium concentration in the injected solutions, measured with a calcium-selective membrane electrode, was $<10^{-7}$ M. Temperature 16 °C.

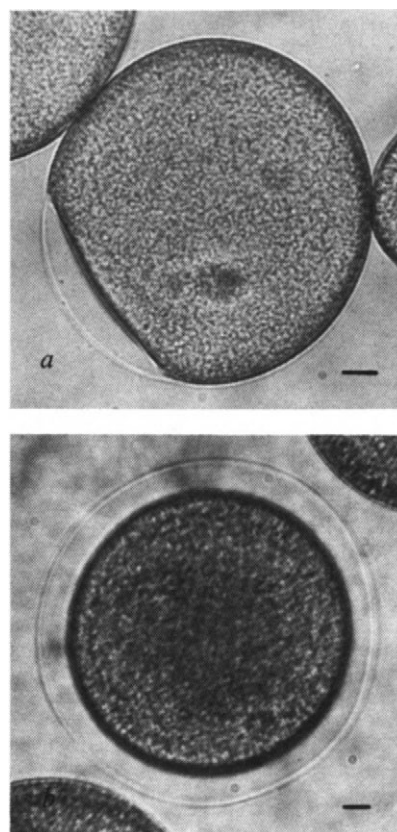


Fig. 2 *a*, A sea urchin egg partially activated by microinjection of 8 pl of 100 nM InsP₃ (8×10^{-7} pmol). Exocytosis has occurred only in a sharply circumscribed region of the egg cortex. *b*, A sea urchin egg wholly activated by microinjection of 0.5 pl of 5 μ M InsP₃ (2.5×10^{-6} pmol). Scale bar, 10 μ m.

evidence that there is a calcium-stimulated breakdown of PtdIns(4,5)P₂ in the egg cortex. We believe that, just as microinjecting InsP₃ stimulates an increase in cytoplasmic calcium, the production of InsP₃ at fertilization may be responsible for the release of calcium from the egg's intracellular store.

The unfertilized sea urchin egg possesses a sensitive *in situ* indicator of cytoplasmic calcium concentration. A cytoplasmic calcium concentration $>1 \mu$ M stimulates exocytosis of the egg's cortical granules^{12,13} and a fertilization envelope appears around the egg. Figure 1*a* shows the amount of InsP₃ required to produce fertilization envelopes when injected into unfertilized eggs. Half the activated eggs had complete fertilization envelopes when 20 pl (4% egg volume) of 100 nM or 4 pl (0.8% egg volume) of 400 nM InsP₃ were injected (2×10^{-6} and 1.6×10^{-6} pmol, respectively). Inositol bisphosphates were less effective: Fig. 1*b* shows that 4 and 5 pl of 25 μ M (1 and 1.25×10^{-4} pmol), or 0.5 pl of 100 μ M (0.5×10^{-4} pmol) Ins(4,5)P₂ were needed to cause elevation of the fertilization envelope; 1 pl of 25 μ M Ins(4,5)P₂ (2.5×10^{-5} pmol) caused partial elevation of the fertilization envelope in 1 of 10 eggs (microinjection of vehicle alone caused activation in 1 of 50 eggs) and 1 pl of 10 μ M Ins(4,5)P₂ (10^{-5} pmol) was ineffective. Injection of 40 pl of 25 μ M (10^{-3} pmol) inositol 1,4-bisphosphate (Ins(1,4)P₂) resulted in the partial elevation of a fertilization envelope in only 1 of 16 eggs. These results indicate that InsP₃ stimulates cortical granule exocytosis at concentrations (assuming even distribution) of the order of 10 nM, substantially lower than those reported to release calcium from membrane stores in permeabilized cells, but with similar specificity⁶⁻⁹. InsP₃ seems to stimulate exocytosis indirectly by increasing the cytoplasmic calcium concentration. We determined that InsP₃ has no direct effect by applying it to isolated portions of egg cortex which do

undergo exocytosis when exposed to physiological calcium concentrations¹³.

InsP₃ releases calcium locally: on injection of 4 pl of 400 nM InsP₃ (1.6×10^{-6} pmol), the fertilization envelope in some eggs rose only over the region of the plasma membrane close to the tip of the micropipette. There is a sharp boundary (Fig. 2) between this area of the plasma membrane and surrounding areas where no exocytosis has occurred. This is consistent with a rapid hydrolysis of InsP₃ in the egg cytoplasm, or with a critical threshold concentration for the release of calcium. Injection of smaller volumes of InsP₃ at higher concentration produced the same result: 0.5 pl of 2.5 μ M InsP₃ (1.25×10^{-6} pmol, an equivalent amount) again produced partial elevation of the fertilization envelope.

When a sperm fertilizes a sea urchin egg, the fertilization envelope first appears at the site of sperm entry and rises progressively over the egg surface, appearing at the antipode about 30 s later¹⁴. The asynchronous elevation of the fertilization envelope reflects an underlying wave of cortical granule exocytosis¹⁵. It has been suggested that the increase in cytoplasmic calcium concentration which occurs at fertilization is not spatially uniform, but propagates from the point of sperm entry¹⁶. Figure 3 shows that microinjection of 0.5 pl of 5 μ M InsP₃ (2.5×10^{-6} pmol) induces a wave of exocytosis which begins at the point on the egg surface nearest the tip of the micropipette and reaches the antipode some 25 s later. This observation could be ascribed as readily to diffusion of the injected substance as to the initiation of a propagated wave of calcium release. Figure 3 also shows, however, that the transit time is constant over a fivefold range of InsP₃ concentrations at constant injection volume and is equivalent to the transit time at fertilization in these conditions; it decreases only at a

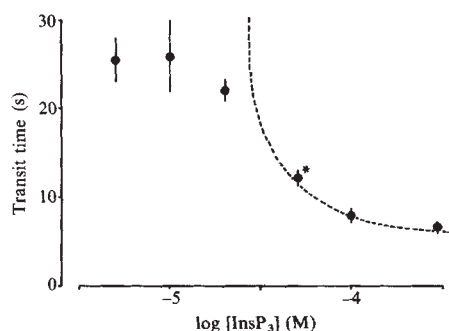


Fig. 3 The dependence of the transit time of the cortical wave of exocytosis on the concentration of InsP_3 microinjected into eggs. The transit time was measured as the time from the first noticeable elevation of the fertilization envelope at the point on the egg cortex nearest the micropipette tip to the time of first noticeable elevation of the fertilization envelope at the point on the egg cortex diametrically opposite the point of initiation. The values are the mean $\pm$ s.e.m. of 20 observations (except for * which is the mean of 15 observations). Volume injected 0.5 μl . Temperature 16 $^{\circ}\text{C}$. The transit time at fertilization at this temperature in this species is 24 ± 1.5 s (mean $\pm$ s.e.m., $n = 15$). The dotted curve represents the expected relation between transit time and injected concentration if the phenomenon were due to diffusion alone. Two assumptions were made: (1) that the diffusion process can be described by the equations of diffusion from a point source into an infinite medium²²; (2) that a critical InsP_3 concentration must be reached before exocytosis occurs. The first is a reasonable approximation because, although the egg is of finite volume, it is probable that first-order degradation of InsP_3 occurs in the egg cytoplasm. The fit is relatively insensitive to these assumptions, always predicting an initial sharp decline in transit time with increased concentration; it is, however, sensitive to the value of the diffusion coefficient. The dotted curve is drawn for a diffusion coefficient of $3.3 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$, a diffusion path length of 70 μm and a critical concentration of 30 nM. This estimate of the diffusion coefficient agrees well with the expected diffusion coefficient for a molecule the size of InsP_3 in cytoplasm²³.

concentration between 20 and 50 μM . The volume of solution injected represents 1/1,000 of the egg volume. These observations are consistent with the notion that InsP_3 induces a propagated wave of calcium release similar to that which may occur at fertilization.

One consequence of the increase in cytoplasmic calcium concentration which occurs at fertilization is an increase in cytoplasmic pH ¹⁷. The cytoplasmic alkalinization is the second of the two ionic signals which cause activation of the egg², and may be detected by measuring the fluorescence of fluorescein trapped in the egg^{11,18}. Microinjection of 0.5 μl of 10 μM InsP_3 (5×10^{-6} pmol) into unfertilized eggs caused a $116 \pm 2.6\%$ (mean $\pm$ s.e.m., $n = 4$) increase in fluorescein fluorescence, similar to the increase of $119 \pm 3.1\%$ (mean $\pm$ s.e.m., $n = 7$) which occurs at fertilization². Thus, we conclude that InsP_3 sets in motion the ionic changes which constitute egg activation.

Our data demonstrate the physiological effects of InsP_3 in an intact cell. In addition, our experiments supply possible answers to the question of how a sperm activates a sea urchin egg. There is a substantial turnover of $\text{PtdIns}(4,5)\text{P}_2$ at fertilization¹¹. Figure 4 shows that the breakdown of $\text{PtdIns}(4,5)\text{P}_2$ in an egg plasma membrane preparation is stimulated by calcium concentrations of 3–5 μM , the concentration range at which exocytosis occurs in this preparation¹³ and also the calcium concentration measured in the egg cytoplasm at fertilization⁴. Our results are consistent with a mechanism in which InsP_3 -stimulated calcium release and calcium-stimulated InsP_3 production cooperate to produce a wave of calcium release which propagates through the egg cytoplasm.

What sets this process in motion? Dale and co-workers have reported that sea urchin eggs can be activated by microinjecting

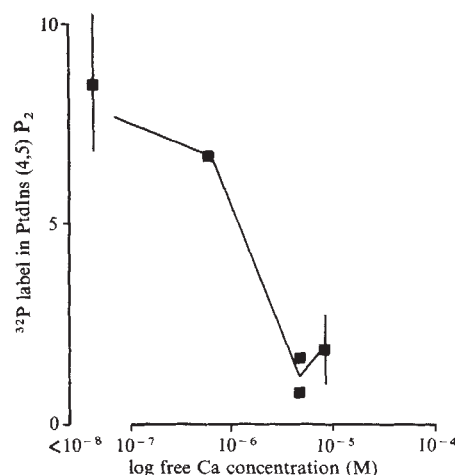


Fig. 4 The calcium dependence of $\text{PtdIns}(4,5)\text{P}_2$ breakdown in cortical fragments isolated from unfertilized sea urchin eggs. A preparation consisting of plasma membrane with associated cortical secretory granules was isolated by attaching eggs to glass coverslips with polylysine and shearing away the bulk of the egg with EGTA-containing medium (220 mM potassium glutamate, 500 mM glycine, 10 mM NaCl, 5 mM MgCl_2 , 2.5 mM ATP, 1 mM EGTA, pH 6.7)¹². This preparation allows direct treatment of the cytoplasmic face of the plasma membrane with experimental solutions. After treatment for 2 min with the above medium containing various amounts of free calcium buffered with EGTA¹², the phospholipids were extracted and separated by chromatography²⁴. Chromatograms were developed on oxalate-treated silica gel 60 TLC plates (Merck, Darmstadt) in chloroform/methanol/acetone/acetic acid/water (40:13:15:12:8)²⁵. Lipids were identified by comparison with standards provided by Dr S. Cockcroft. Eggs incubated for 2–3 h in artificial seawater¹² in the presence of ^{32}P -orthophosphate (200 $\mu\text{Ci ml}^{-1}$, 50% v/v suspension; Amersham) incorporated lipid-associated ^{32}P predominantly into $\text{PtdIns}(4,5)\text{P}_2$ and phosphatidylinositol-4-phosphate ($\text{PtdIns}(4)\text{P}$). The radioactive spots corresponding to $\text{PtdIns}(4,5)\text{P}_2$ were identified by autoradiography, scraped from the plate and measured by liquid scintillation counting. The amount of $\text{PtdIns}(4,5)\text{P}_2$ associated with the cortical fragments is expressed as a percentage of the radioactive $\text{PtdIns}(4,5)\text{P}_2$ present in 2 μl of packed eggs. By labelling egg surface proteins with fluorescein isothiocyanate, we have determined that the amount of egg cortex used for each experimental point corresponds to the cortex of 0.14 μl of packed eggs. It seems, therefore, that little breakdown of $\text{PtdIns}(4,5)\text{P}_2$ occurs when the preparation is made in the presence of excess EGTA. The points without error bars represent single determinations. $\text{PtdIns}(4,5)\text{P}_2$ associated with the cortex in low calcium medium ($<10^{-8}$ M calcium) is $8.6 \pm 2.09\%$ (as above) (mean $\pm$ s.e.m., $n = 9$); the amount associated with the cortex 2 min after treatment with 8 μM calcium is $1.9 \pm 0.81\%$ (as above) (mean $\pm$ s.e.m., $n = 8$). This difference is significant ($P < 0.01$, Student's two-tailed t -test). Temperature 16 $^{\circ}\text{C}$.

the soluble fraction of a sperm homogenate¹⁹. Eggs can be activated by microinjecting micromolar calcium²⁰ but the soluble sperm fraction is reportedly calcium-free. It is an attractive possibility that the sperm introduces InsP_3 or a phospholipase C activity on fusion with the egg, triggering the autocatalytic cycle of calcium release and $\text{PtdIns}(4,5)\text{P}_2$ hydrolysis.

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Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells

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The first step in the induction of immune responses, whether humoral or cell mediated, requires the interaction between antigen-presenting cells and T lymphocytes restricted at the major histocompatibility complex (MHC)^{1,2}. These cells invariably express MHC class II molecules (HLA-D region in man and Ia in mouse) which are recognized by T cells of the helper/inducer subset in association with antigen fragments^{3,4}. Interestingly, in certain pathological conditions^{5–8}, for example in autoimmune diseases such as thyroiditis⁵ and diabetic insulinitis⁶, class II molecules may be expressed on epithelial cells that normally do not express them. We speculated that these cells may be able to present their surface autoantigens to T cells, and that this process may be crucial to the induction and maintenance of autoimmunity⁹. A critical test of this hypothesis would be to determine whether epithelial cells bearing MHC class II molecules (class II⁺ cells) can present antigen to T cells. We report here that class II⁺ thyroid follicular epithelial cells (thyrocytes) can indeed present viral peptide antigens to cloned human T cells.

We have described recently a T-cell clone (HA1.7) that is specific for a defined peptide (p20; residues 306–329) of the HA-1 molecule of influenza strain A haemagglutinin (subtype H₃N₂)¹⁰. In the presence of antigen-presenting cells, these T cells proliferate in response to the intact influenza virus of the appropriate subtype. As the antigenic determinant recognized by HA1.7 (p20) is also present in the intact virus, these cloned T cells provide a sensitive and homogeneous assay for the capacity of other cell types to function as antigen-presenting cells. However, the induction of T-cell proliferation to either the intact virus or p20 requires recognition of the relevant MHC class II polymorphism (for HA1.7 this is an HLA-DC/DS (now termed HLA-DQ) specificity in linkage disequilibrium with HLA-DR1; ref. 11). It was essential, therefore, to select potential antigen-presenting cell populations from a histocompatible donor.

We used epithelial thyroid cells from a patient with Graves' disease (hyperthyroidism), because this disorder is commonly treated by surgery, and thyroidectomy glands are a good source of MHC class II⁺ thyrocytes. Peripheral blood mononuclear leukocytes (PBL) from the same thyroid donor (with the

appropriate HLA-DR phenotype) were assayed for their ability to present antigen to clone HA1.7. Irradiated PBL induced substantial proliferative responses of HA1.7 to either influenza A/Texas virus or p20 (Fig. 1). Viable thyrocytes from the same donor, after the removal of non-adherent cells, were stained and found to be positive for MHC class II molecules. They were identified as thyroid epithelial cells by staining with Hashimoto's serum, known to contain antibodies to the microvillar/microsomal antigens expressed on thyrocytes¹². No contaminant macrophages were detected using monoclonal antibodies, thus excluding the possibility that they were acting as antigen-presenting cells. The percentage of DR⁺ thyrocytes could not be determined precisely, because cells were in confluent monolayers, but fluorescence microscopy showed it to be in the range of 60–80%.

When these thyrocytes were tested for their capacity to present intact influenza virus and p20 to T cells of clone HA1.7, there was a reproducible marked proliferative response to p20. This effect was also tested over a limited dose range of thyrocytes (10³ or 10⁴) (Fig. 1), because in certain circumstances the number of antigen-presenting cells is critical¹³. Unlike the results obtained with the PBL from the thyroid donor (Fig. 1), there was no response to whole formalin-inactivated A/Texas influenza virus (Fig. 2) over a wide dose range.

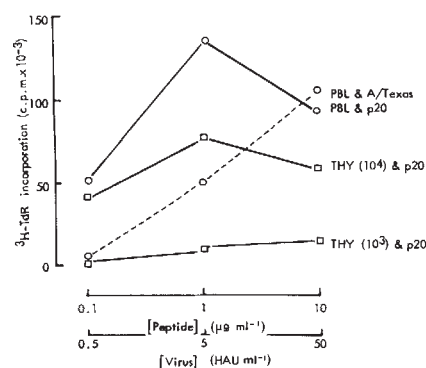


Fig. 1 Presentation of intact influenza virus and peptide to cloned T cells by histocompatible peripheral blood leukocytes from a patient with Graves' disease.

Methods: T-lymphocyte clones specific for synthetic peptides of influenza haemagglutinin (HA) were isolated as previously described. Briefly, PBL were cultured for 6 days with 0.1 μg ml⁻¹ HA (gift of Dr R. G. Webster). Following enrichment on a discontinuous Percoll (Pharmacia) gradient, lymphoblasts were resuspended in RPMI 1640 (Gibco) containing 10% A⁺ serum and T-cell growth factor (TCGF) and plated at one cell every third well in Microtest II trays (Falcon) with 10⁴ irradiated autologous PBL and 0.1 μg ml⁻¹ HA. After 7 days, growing clones were transferred to 96-well microtitre trays and then to 24-well trays. At each transfer the clones received fresh TCGF and autologous irradiated PBL together with specific antigen. The clones were expanded in 25-cm² tissue culture flasks receiving irradiated PBL and intact virus (A/Texas/1/77) every 7 days and fresh TCGF every 3–4 days. TCGF was prepared from 48-h supernatants of PBL (1 × 10⁶ ml⁻¹) cultured with 0.1% purified phytohaemagglutinin (Difco) in complete culture medium containing 2.5% A⁺ serum. Before use in proliferation assays, the clones were rested 6–7 days after the addition of irradiated filler cells. For proliferation assays¹⁸, cloned T cells (5 × 10⁴ ml⁻¹) were cultured with irradiated PBL (20 × 10⁴ ml⁻¹) in the presence of p20 (0.1–10 μg ml⁻¹, ○—○), A/Texas (0.5–50 HAU ml⁻¹, ○---○) or thyrocytes (10⁴ or 10³, □—□) in the presence of p20 0.1–10 μg ml⁻¹. Following 60 h incubation, the cultures were pulsed for 8–16 h with 1.0 μCi ml⁻¹ of ³H-thymidine (TdR, Amersham) and collected onto glass fibre filters. Proliferation as correlated with ³H-TdR incorporation was measured by liquid scintillation spectroscopy. Results are expressed as mean counts per min for triplicate cultures. Background response of cloned T cells alone was 271 ± 5 and in the presence of IL-2 it was 25,672 ± 729 (arithmetic mean ± s.d.).

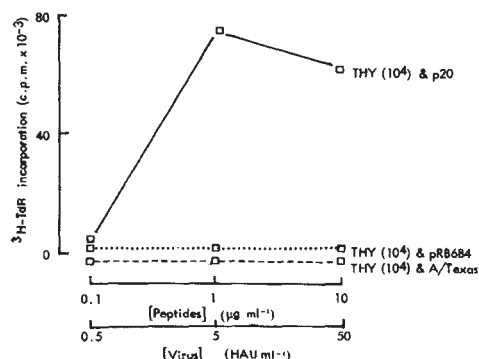


Fig. 2 Response of HA1.7 to inactivated influenza virus and p20 in the presence of histocompatible thyrocytes.

Methods: Irradiated (3,000 rad) thyrocytes from the same donor as for the PBL were prepared by collagenase type IV (Worthington) digestion at 37 °C (5 mg ml⁻¹) for 3 h as described in more detail elsewhere¹⁹, in the absence of any serum. The thyrocytes (10⁴ per well) were plated in 96-well flat bottom microtitre trays and after 1 day, washed to remove any non-adherent cells that may be able to present antigen. They were then cultured with T-lymphocyte clones specific for synthetic peptide of influenza haemagglutinin (HA1.7)¹⁰, in the presence of p20 (0.1–10 μg ml⁻¹, □—□) or formalin-inactivated influenza A/Texas (0.5–50 HAU ml⁻¹, □---□). A non-stimulatory influenza peptide of the HA-1 molecule (RB6B4, residues 314–329) was used as a control (□···□). Proliferation was measured by thymidine incorporation, as described in Fig. 1 legend. The degree of macrophage/monocyte contamination was checked using the monoclonal antibodies TG1 and UCHM1 (ref. 20). The response of HA1.7 alone was 114 ± 77, and in the presence of IL-2 it was 40,813 ± 4,930 (arithmetic mean ± s.d.).

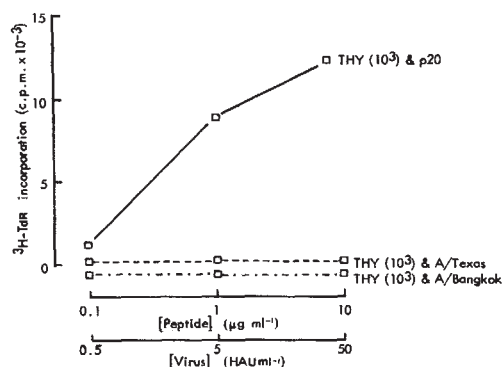


Fig. 3 Response of HA1.7 to infectious inactivated influenza virus and p20 to the presence of histocompatible thyrocytes.

Methods: Cloned T cells (HA1.7) were cultured with infectious influenza A/Bangkok (0.5–50 HAU ml⁻¹, □···□), inactivated influenza A/Texas (0.5–50 HAU ml⁻¹, □---□) or p20 (0.1–10 μg ml⁻¹, □—□) in the presence of irradiated thyrocytes (10³ per well). The background response of HA1.7 alone or in the presence of IL-2 were, respectively, 393 ± 259 and 22,417 ± 7,839.

Table 1 Inhibition of thyrocyte-induced response to p20 by anti-class II antibody

Cells	Antigen	Antibody	Response (c.p.m. ± s.d.)
HA1.7	—	—	114 ± 77
HA1.7	—	IL-2	40,813 ± 4,930
HA1.7	Thyrocytes	RB6B4	1,110 ± 571
HA1.7	Thyrocytes	p20	75,041 ± 14,844
HA1.7	Thyrocytes	p20	3,460 ± 610
HA1.7	—	p20	1,112 ± 90
—	Thyrocytes	—	142 ± 26

Cloned T cells (10⁴ per well) were cultured with MHC class II⁺ irradiated (3,000 rad) thyrocytes (10⁴ per well) in the presence of p20 or RB6B4 (1 μg ml⁻¹). Anti-HLA class II ascites (HIG78)²¹, which is known to block the response of HA1.7 (ref. 11), was included from the start of the cultures, at a dilution of 1/200. Proliferation was determined as described in Fig. 1 legend. Control responses of HA1.7 alone or with added interleukin-2 (IL-2) are shown.

manuscript in preparation). It seemed unlikely that monocyte/macrophage-like cells in the thyrocyte preparations were involved, because these were not detected by staining with monoclonal antibodies.

As thyrocytes failed to present formalin-inactivated A/Texas virus (Figs 2, 3), it was of interest to determine whether they were able to present an infectious preparation of A/Bangkok, which contains p20 of identical sequence to that of influenza A/Texas. The thyrocytes did not present infectious virus (Fig. 3), but did present p20 to HA1.7. The inability of MHC class II⁺ thyrocyte preparations to present either infectious or inactivated influenza virus, in contrast to PBL, indicates that the antigen presentation noted could not have been due to any undetected contaminating MHC class II⁺ PBL. Although it was conceivable that the failure of thyrocytes to present influenza A virus was due to their inability to bind and take up influenza virus, this seemed unlikely, as influenza virus, which uses neuraminic acid as cellular receptor, can infect virtually all cell types¹⁷. This possibility was excluded by the strong staining of influenza A virus-incubated thyrocytes with rabbit anti-influenza A/Bangkok antiserum.

To verify that the presentation of p20 by thyrocytes was an immunologically specific reaction and not a mitogenic effect perhaps due to thyroid hormones, another peptide (RB6B4) from the same virus sequence but which does not activate HA1.7, was used as antigen, without effect (Fig. 2). To confirm that the expression of MHC class II antigen by thyrocytes was essential to their presenting function, an anti-class II monoclonal antibody, which inhibits the response of HA1.7 to PBL used as antigen-presenting cells¹¹, was used in co-culture experiments. It efficiently inhibited the response of HA1.7 to p20 which was induced by thyrocytes acting as antigen-presenting cells (Table 1).

The capacity of thyrocytes to present peptide antigen clearly indicates the functional nature of the MHC class II molecules detected on the surface of thyrocytes by immunofluorescence with monoclonal antibodies, and thus we can anticipate that other cells expressing aberrant HLA-DR or Ia molecules in other disease states^{6–9} may in the appropriate circumstances act as autoantigen-presenting cells. The restriction on the type of antigen presented by DR⁺ thyroid epithelial cells is noteworthy. These cells were capable of presenting only peptides which did not need further catabolism or 'processing' in order to expose their antigenic determinants, but could not present intact virus, which requires processing. This suggests that, *in vivo*, class II⁺ epithelial cells may be able to present all antigens, but only those that do not need further alteration. Thus, surface autoantigens already expressed in the plasma membrane could be efficiently presented to potentially autoreactive T cells. Indeed, we have demonstrated recently that autoreactive T cells, cloned from the intra-thyroid lymphocytes of autoimmune thyroiditis, are re-stimulated by their autologous MHC class II⁺ thyrocytes,

After 24 hours the thyrocyte cultures were vigorously washed 3 times prior to the addition of T cells and antigen, in order to remove non-adherent cells, confirming that the presenting capacity was due to adherent thyrocytes and not to non-adherent MHC class II⁺ cells such as B cells or activated T cells. Dendritic cells, which are important antigen-presenting cells in some proliferative responses, are adherent at 2 h but not at 18 h, and thus would have been expected to be removed by this procedure^{14,15}. Furthermore, we have found recently that mouse dendritic cells, unlike human thyrocytes, can present particulate antigens such as red cells and mycobacteria¹⁶ and can process proteins such as keyhole limpet haemocyanin (B. Chain, P. Kaye & M.F.,

but not by histoincompatible thyrocytes (M.L., G.F.B. and M.F., manuscript submitted).

The restricted antigen-presenting function of class II⁺ epithelial cells is compatible with the need for specialized antigen-presenting cells, such as macrophages¹ and dendritic cells¹⁴ which can process^{3,16} antigen, in generating immune responses to foreign antigens such as viruses. These experiments strongly support the concept that aberrant MHC class II expression is important in perpetuating, and perhaps initiating autoimmune reactions⁹, and indicates that control of this expression may be of importance in abrogating autoimmunity.

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Human interleukin-2 promotes proliferation of activated B cells via surface receptors similar to those of activated T cells

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Human interleukin-2 (IL-2) is a glycoprotein of relative molecular mass (M_r) 15,000, which is released by T lymphocytes on stimulation with antigen or mitogen and functions as a T-cell growth factor (TCGF) by inducing proliferation of activated T cells¹. It is generally accepted that resting or activated B cells do not respond directly to IL-2 but require for their proliferation other T-cell-derived lymphokines usually referred to as B-cell growth factors (BCGFs)^{2,3}. Recently, however, a monoclonal antibody reacting

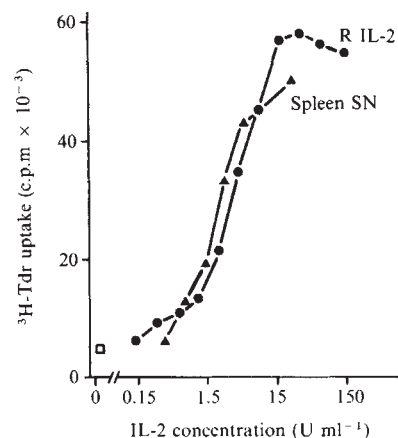


Fig. 1 B-cell growth factor activity of human IL-2. BCGF activity was measured using the assay system described by Muraguchi and Fauci⁹, with minor modifications¹⁰. This system is based on culturing B cells for 72 h with formaldehyde-killed *S. aureus* Cowan strain I before addition of tested growth factors. The *S. aureus* was grown in trypticase soy broth, inactivated by formaldehyde and used at a final dilution of 1:4,000. B-cell populations were represented by non-adherent spleen lymphocytes that had been depleted twice of cells forming rosettes with neuraminidase-treated sheep erythrocytes as described elsewhere¹⁶. The resulting cells contained <1% erythrocyte-rosetting cells. Further analysis of this population was performed using FACS (see Fig. 2). These B cells (5×10^4) were cultured in round-bottomed microtitre trays in 0.2 ml RPMI 1640 with 10% fetal calf serum (FCS) in the presence or absence of *S. aureus*. Serial dilutions of standard spleen supernatant (▲—▲) or recombinant IL-2 (●—●) were added at day 3. Cultures were continued for an additional 72 h and 0.5 μ Ci of tritiated thymidine (³H-TdR) was added 18 h before collection and measurement of thymidine incorporation. As an indicator cell system for the determination of IL-2 biological (TCGF) activity, we used the CTLL murine cytolytic T lymphocyte line known to proliferate in response to human IL-2 (but not to PHA)¹. These cells were cultured for 24 h in Dulbecco's modified Eagle's medium and 5% FCS in the presence of serial dilutions of tested supernatant. IL-2 activity was assessed by ³H-TdR uptake after 24 h, following a 6-h pulse with 0.5 μ Ci ³H-TdR. The value of 1 U ml⁻¹ was assigned to an IL-2 concentration leading to 50% maximal ³H-TdR uptake. The recombinant human IL-2, purified and free of endotoxin (specific activity 1 U per 0.3 ng protein), was provided by Biogen. The cloning and screening of human IL-2 cDNA plasmid obtained from RNA isolated from PHA-stimulated human spleen cells and its expression in *E. coli* have been performed by Devos *et al.*⁸.

with the IL-2 receptor molecules expressed by activated T cells (anti-Tac)^{4,5} was shown to react also with certain B tumour cells⁶; in addition, murine B cells proliferate in response to pure human IL-2⁷. We now show that recombinant IL-2, derived from *Escherichia coli* expressing the human gene⁸, is able to promote strong proliferation of human B cells activated with protein-A-rich *Staphylococcus aureus* Cowan strain I². Moreover, we demonstrate that the anti-Tac antibody also reacts with *S. aureus*-activated normal B cells and inhibits sharply the proliferative response of such cells to IL-2. Finally, immunoprecipitation experiments reveal that anti-Tac defines similar molecules on activated T and B cells.

BCGF activity is measured by an assay system described previously⁹, with minor modifications. This system is based on the growth factor-induced proliferative response of B cells pre-activated for 3 days with an appropriate source of formaldehyde-killed protein A-rich *S. aureus* Cowan strain I. Cell proliferation is evaluated by ³H-thymidine incorporation after an additional 3-days' culture. The ability of recombinant IL-2 to promote T- or B-cell proliferation is compared with that of standard phytohaemagglutinin (PHA)-induced spleen supernatant, routinely used in our laboratory as a source of both TCGFs and BCGFs¹⁰. First, we determined the TCGF activity of the samples examined by a proliferation assay using the CTLL murine cytolytic T-lymphocyte line, known to proliferate in response

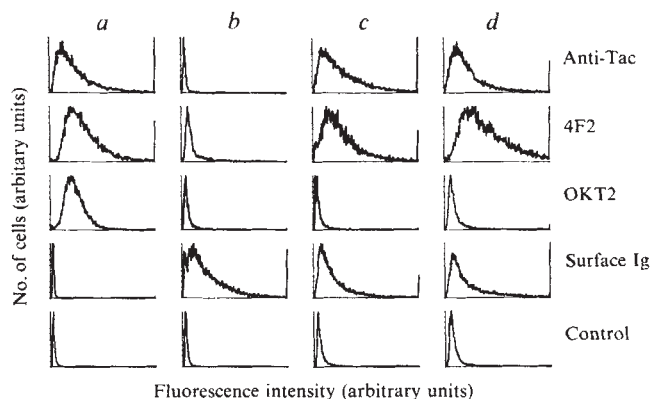


Fig. 2 Anti-Tac antibody binds to activated B cells. Human spleen B cells, isolated as described in Fig. 1 legend, were analysed for surface antigen expression either directly (*b*), or after 3 days of activation with *S. aureus* (*c*) or after an additional 3 days of culture in the presence of 15 U ml^{-1} recombinant IL-2 (*d*) (see Fig. 1). T cells that had been stimulated for 3 days with 1% PHA and then cultured for a further 3 days with recombinant IL-2 (15 U ml^{-1}) were analysed for comparison (*a*).

Methods: 2×10^5 cells were incubated with one of the following monoclonal antibodies: anti-Tac (100 μl of 1:10,000 ascites in medium), 4F2 (ref. 11) (100 μl of 1:2,000 ascites in medium), OKT3 (5 μl of the standard solution). Control cells were incubated in culture medium with no antibody. Cells were washed and incubated with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin for 30 min¹⁵. To detect surface immunoglobulin, cell samples were incubated with the F(ab')_2 of a goat anti-human immunoglobulin¹⁰. Samples were then washed and analysed on a BD FACS system as described previously^{15,17}. Note that both activated B-cell populations expressed the Tac (or the 4F2) antigen to a level similar to that of activated T cells; in contrast, no Tac (or 4F2) antigen was detectable in resting B cells. The B-cell nature of the *S. aureus*-activated cell population was indicated by the absence of T3⁺ cells by the expression of surface immunoglobulin.

to human IL-2¹. Serial dilutions of these samples were then tested for BCGF activity in the *S. aureus*-activated B-cell assay. B-cell proliferation was promoted by both sources of IL-2 (Fig. 1) and we found a strong correlation between BCGF and TCGF activities of the two samples. We examined whether proliferation of contaminating T lymphocytes could account for the response to IL-2 observed in the activated B-cell assay. T lymphocytes were counted as cells forming rosettes with sheep erythrocytes and/or as T3-positive cells by analysis using fluorescein-activated cell sorting (FACS). There were always <1% erythrocyte-rosetting lymphocytes in the cell populations tested before culture and <2% at the end of culture (six different experiments); in addition, T3⁺ cells could not be detected by flow microfluorometry (see also Fig. 3). Thus, we conclude that the ³H-thymidine incorporation observed in *S. aureus*-activated B-cell populations after exposure to IL-2 reflects proliferation of B cells and not contaminating T cells.

Given the ability of recombinant IL-2 to induce proliferation of activated B cells, it was of interest to determine whether binding of IL-2 to B cells occurred via surface receptors similar to those expressed by activated T cells. B cells activated by *S. aureus* were therefore analysed for their reactivity with the anti-Tac monoclonal antibody, which is specific for the IL-2 receptors expressed by activated T cells^{4,5}. Previous studies indicated that other surface molecules expressed by activated T cells, such as the 120,000- M_r 4F2, were also expressed by B cells following activation with *S. aureus*¹¹. The expression of Tac and 4F2 antigens was assessed by indirect immunofluorescence and flow microfluorometry on B cells either 3 days after activation with *S. aureus* or after an additional 3-days' culture in the presence of recombinant IL-2. Fresh unstimulated B cells and PHA-activated T cells were analysed for comparison. Both

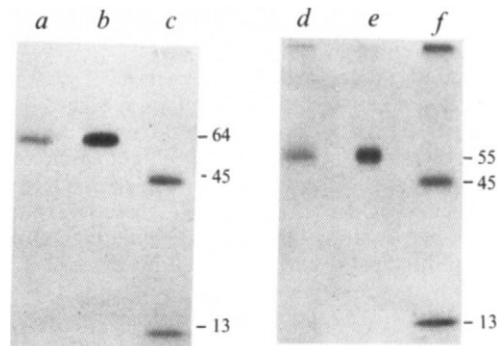


Fig. 3 Anti-Tac antibody recognizes similar molecules on activated T or B cells. *a*, *b*, Electrophoretic patterns obtained following immunoprecipitation, in reducing conditions, of ¹²⁵I-labelled B and T cells, respectively, with anti-Tac monoclonal antibody. *d*, *e*, The same molecules immunoprecipitated in non-reducing conditions. Migration of HLA class I molecules immunoprecipitated from *S. aureus*-activated B cells with the B9.12 antibody in reducing (*c*) or non-reducing (*f*) conditions is shown for comparison. Note that the apparent M_r of Tac antigen in reducing conditions is ~64,000 in both cell types, while the M_r in non-reducing conditions is 55,000, thus providing evidence for the existence of internal disulphide bond(s) in both T and B cells.

Methods: Spleen B cells were cultured with *S. aureus* for 3 days (see Fig. 1) and with recombinant IL-2 (15 U ml^{-1}) for an additional 48 h. Cells from several microwell plates were pooled and surface labelled with ¹²⁵I using lactoperoxidase-glucose-oxidase-catalysed iodination¹². T cells cultured for 3 days with 1% PHA and for an additional 3 days with recombinant IL-2 were also surface-labelled. Approximately 20×10^6 cells were washed and resuspended in 1 ml phosphate-buffered saline (PBS) containing glucose (5.5 mM) and the following reagents: 20 μl KI ($5 \times 10^{-5} \text{ M}$), Na^{125}I (1 mCi), 40 μl lactoperoxidase (1 mg ml^{-1} solution in PBS), 10 μl glucose oxidase (stock solution diluted 1:100 in PBS). Cells were incubated at 4°C. After 10 min and 20 min, 10 μl glucose oxidase were added and the incubation was carried out for a total period of 30 min. Cells were then washed five times in cold RPMI, twice in PBS and lysed in 10 mM Tris-buffered saline (pH 7.5) containing 1% NP40, 1 mg ml^{-1} bovine serum albumin and 0.1 mM phenylmethylsulphonyl fluoride (PMSF) for 20 min at 4°C. The supernatant of a 30-min spin at 100,000g was then immunoprecipitated as follows. Supernatants were dialysed with PBS containing 0.05% NP40 and 0.1 mM PMSF and precleared twice with 100 μl of packed protein-A-Sepharose beads for 2 h under rotation. Aliquots (200 μl) were then incubated for 2 h with 10 μl of a 1:10 dilution of anti-Tac or B9.12 (anti-HLA)¹⁸ ascites. 20 μl of protein-A-Sepharose beads were added and samples incubated for 2 h at 4°C. The immunoprecipitate was eluted from protein-A-Sepharose by boiling for 2 min in 5% SDS in the presence or absence of 5% 9-mercaptoethanol and analysed on 11% discontinuous SDS-polyacrylamide gels¹⁹.

activated B-cell populations expressed the 4F2 and Tac antigens to a similar level to that observed on activated T cells (Fig. 2). On the other hand, neither antigen was detected on unstimulated B cells. That the cells expressing Tac antigen in the *S. aureus*-activated cell populations were indeed B cells (and not T cells) was confirmed by the demonstration that most cells expressed surface immunoglobulins whereas T3⁺ cells were virtually absent.

In view of the strong reactivity of anti-Tac with activated T and B cells, we then compared the anti-Tac-reactive molecules present in the two lymphocyte populations. B cells stimulated with *S. aureus* and PHA-induced T-cell blasts were surface labelled with ¹²⁵I by the lactoperoxidase-glucose oxidase-catalysed iodination method¹². They were then lysed and the resulting cell extracts immunoprecipitated with anti-Tac. On SDS polyacrylamide gel electrophoresis (PAGE), the molecules recognized by anti-Tac in both lysates displayed a M_r of ~55,000 in nonreducing conditions (Fig. 3, lanes *a*, *b*) and 64,000 in reducing conditions (lanes *d*, *e*). We therefore conclude that the molecules serving as IL-2 receptors on activated T and B cells are similar.

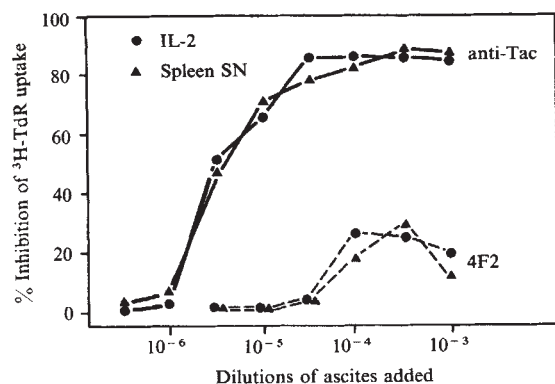


Fig. 4 Anti-Tac inhibits the IL-2-induced proliferation of *S. aureus*-activated B cells. Serial dilutions of anti-Tac or anti-4F2 were added to 5×10^4 spleen B cells that had been cultured for 3 days with optimal concentrations of *S. aureus* in round-bottomed 96-well microtitre plates. Spleen supernatant or recombinant IL-2 were added subsequently at a final concentration of 7 U ml^{-1} IL-2. Triplicate cultures were incubated for 72 h at 37°C and 0.5 mCi of $^3\text{H-TdR}$ was added 18 h before collection (see also Fig. 1). Data are expressed as % inhibition of the response obtained in cultures receiving spleen supernatant or recombinant IL-2 alone ($53,500 \pm 3,400 \text{ c.p.m.}$ and $46,800 \pm 1,400 \text{ c.p.m.}$, respectively).

We next investigated whether the IL-2-induced B-cell proliferative response could be inhibited by anti-Tac (in the absence of complement). The B-cell proliferative response to recombinant IL-2 or spleen supernatant was strongly inhibited by the addition of anti-Tac at a final dilution of 10^{-5} of anti-Tac ascitic fluid whereas anti-4F2 ascites, used as control, had minimal inhibitory effect even when added at final dilutions as high as 10^{-3} (Fig. 4). According to previous studies, supernatant of lectin-stimulated lymphocytes should contain lymphokines with BCGF activity in addition to IL-2. However, in our study, up to 90% inhibition of B-cell proliferation was achieved with the anti-Tac. Taken together, the present results suggest that IL-2 may be responsible for a large part of the BCGF activity generated by PHA-stimulated human spleen cells. Our data do not rule out the possibility that molecules other than IL-2 display BCGF activity. For example, supernatants of lectin-stimulated 72-h lymphocyte cultures have been reported to contain strong BCGF activity but little TCGF activity²; in addition, T-T-cell hybridomas have been described which released BCGF and not IL-2^{13,14}. In any case, limiting dilution analysis of the precursors of IL-2-producing cells has previously indicated that as many as 60% of peripheral blood T cells have this functional potential¹⁵, and it is therefore evident that a large fraction of human T cells can also influence B-cell responses via IL-2.

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Production of functional chimaeric mouse/human antibody

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The availability of monoclonal antibodies has revived interest in immunotherapy. The ability to influence an individual's immune state by administering immunoglobulin of the appropriate specificity may provide a powerful approach to disease control and prevention. Compared with immunoglobulin from other species, human immunoglobulin (Ig) might be best for such therapeutic intervention; it might function better with the recipient's effector cells and should itself be less immunogenic. The success of the mouse hybridoma system suggests that immunoglobulin of virtually any specificity can be obtained from a properly immunized animal. In the human system, however, immunization protocols are restricted by ethical considerations, and it is not yet clear whether human antibody-producing cell lines of the required specificity can be obtained from adventitiously immunized individuals or from *in vitro* immunized cells. A method which might circumvent these difficulties is to produce antibodies consisting of mouse variable regions joined to human constant regions. Therefore, we have constructed immunoglobulin genes in which the DNA segments encoding mouse variable regions specific for the hapten trinitrophenyl (TNP) are joined to segments encoding human μ and κ constant regions. These 'chimaeric' genes are expressed as functional TNP-binding chimaeric IgM. We report here some of the properties of this novel IgM.

The variable regions used in the experiments described here are derived from the hybridoma cell line Sp6 which secretes IgM(κ) specific for TNP¹. The specific κ gene² and μ gene³ have been cloned and were used as a source of TNP-specific variable regions which were joined to cloned human μ and κ constant regions^{4,5} in the vector pSV₂-neo⁶ (Fig. 1).

To assay the chimaeric light- and heavy-chain genes independently of each other, we transferred these genes into appropriate mutant hybridoma cell lines derived from Sp6. The vector pN· χ - κ TNP bearing the chimaeric κ light-chain gene, was transferred as described elsewhere⁷⁻⁹ to the mutant cell line, igk14 (ref. 7). This cell line has lost the ability to produce the TNP-specific κ light chain (κ_{TNP}) but continues to synthesize the TNP-specific μ heavy chain (μ_{TNP}). In a similar manner, the vector pN· χ - μ TNP bearing the chimaeric heavy-chain gene, was transferred to another mutant cell line, igm10 (ref. 3), which produces κ_{TNP} but no μ_{TNP} . To produce the totally chimaeric IgM, we transferred the vectors pN· χ - κ TNP and pN· χ - μ TNP together into the cell line, Sp2/0 (ref. 10), which produces neither heavy nor light immunoglobulin chains. Transformants were selected for resistance to the drug G418, then tested for their production of TNP-specific IgM, as measured by their ability to agglutinate TNP-coupled sheep red blood cells (TNP-SRBC). The frequency at which stable G418-resistant transformants were generated was found to be 10^{-3} . In experiments involving a single vector-transfer, approximately 30% of the resistant transformants produced detectable IgM. In the co-transfer experiment, where the chimaeric κ and μ were introduced into Sp2/0, we estimate that 0.1-1% of the transformants produced enough of both μ_{TNP} and κ_{TNP} to make detectable IgM. The selective advantage to co-transfer seems here to be significantly less than that reported by others using calcium phosphate coprecipitation¹¹ or protoplast fusion¹². We have also transferred the chimaeric heavy- and light-chain genes sequentially: the heavy-chain vector, pN· χ - μ TNP, was first introduced into

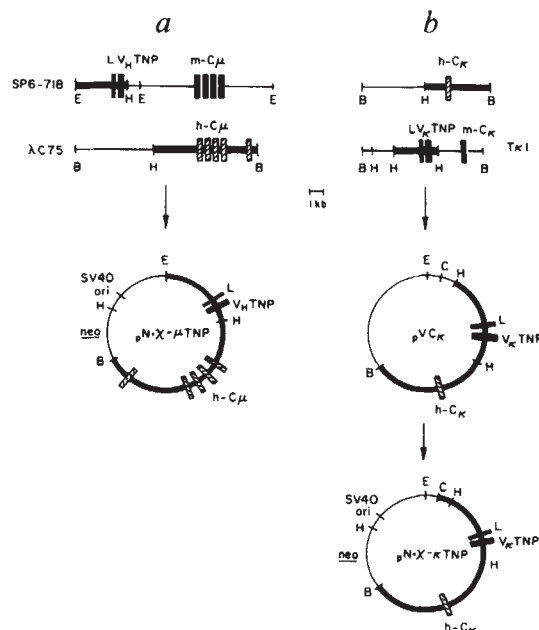


Fig. 1 *a*, Construction of the chimaeric heavy-chain gene. The 3.5-kilobase (kb) *EcoRI*-*HindIII* fragment containing the mouse TNP-specific heavy-chain variable region was obtained from the cloned TNP-specific heavy chain, Sp6-718 (ref. 3). This fragment, together with the 8.0-kb *BamHI*-*HindIII* fragment containing the human constant region from the cloned segment λ C75 (ref. 4), was introduced into pSV2-neo by ligation at the *EcoRI*-*BamHI* site. *b*, Construction of the chimaeric light-chain gene. The 5.4-kb *BamHI*-*HindIII* fragment containing the human C_{κ} gene was introduced into pVR322 by ligation at the *BamHI*-*HindIII* site. The 4.2-kb *HindIII*-*HindIII* fragment containing the mouse TNP-specific light-chain variable region was obtained from the cloned TNP-specific light chain $T_{\kappa}1$ (ref. 2) and introduced into the *HindIII* site of the pVR322 vector containing the human C_{κ} . From this plasmid, pVC κ , the 9.6-kb *BamHI*-*Clal* fragment containing the chimaeric light chain was obtained and introduced at the *BamHI*-*Clal* site of a modified pSV2-neo vector containing the *BamHI*-*EcoRI* segment derived from pVR322. The chimaeric light-chain gene was also introduced into the vector pSV2-gpt¹³ in the manner described above. Restriction enzymes: B, *BamHI*; C, *Clal*; E, *EcoRI*; H, *HindIII*.

Sp2/0; in a subsequent step, transfer of the light-chain gene, carried in this case on the vector pSV2-gpt¹³, was selected by resistance to mycophenolic acid. Stable transformants which produced the highest levels ($\sim 5 \mu\text{g ml}^{-1}$) of IgM were selected for further study and cloned by limiting dilution. The vector pR-HLTNP, which bears in its entirety the mouse genes for TNP-specific μ and κ chains, was also transferred to the cell line Sp2/0, (refs 3, 14) and the IgM made by one such transformant, TSp2/mIgM12, is compared here with the chimaeric IgM made by the transformant TSp2/ χ -IgM1. By using various antisera specific for antigenic determinants of the mouse and human μ and κ constant regions, we confirmed that the chimaeric genes encode chimaeric proteins, that is, the TNP-binding capacity of the mouse IgM is linked to antigenic determinants of human μ and κ chains (results not shown).

To determine whether the chimaeric IgM is pentameric, we analysed the biosynthetically labelled IgM secreted by these transformants using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-denatured chimaeric IgM from TSp2/ χ -IgM1 migrates at nearly the same rate as pentameric mouse IgM produced by the hybridoma Sp6 and the transformant TSp2/mIgM12 (Fig. 2A), indicating that the chimaeric genes produce μ and κ chains which combine to form pentameric IgM. After reduction of the disulphide bonds, chimaeric μ and κ chains migrate slightly slower than the corresponding mouse chains (Fig. 2B). The molecular weights of the human μ and

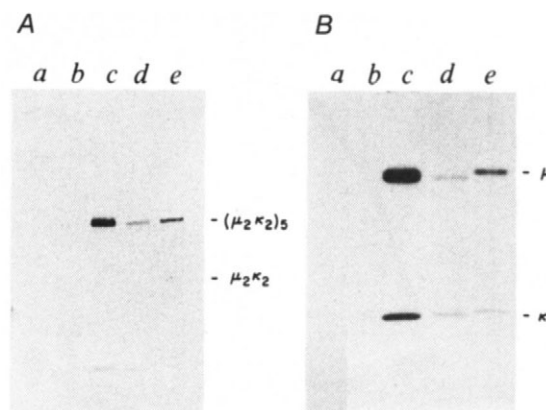


Fig. 2 Production of immunoglobulin heavy and light chains in transformants. The transformants expressing the chimaeric μ and κ genes are compared with two cell lines making TNP-specific murine IgM: the parental Sp6 hybridoma (Sp603 subclone) from which the μ_{TNP} and κ_{TNP} genes were cloned, and a transformant (TSp2/mIgM12) expressing the transferred murine μ_{TNP} and κ_{TNP} genes (denoted as cell line Sp2/T12 in ref. 14). The secreted IgM(κ) of the indicated cell lines was biosynthetically labelled with ^{14}C -leucine and subjected to SDS-PAGE as described²³. Immunoglobulin from cell lines Sp2/0 (*a*), Sp6 (*c*) and TSp2/mIgM12 (*d*) was precipitated with anti-mouse IgM. Immunoglobulin from the cell lines Sp2/0 (*b*) and TSp2/ χ -IgM1 (*e*) was precipitated with anti-human IgM. *A*, Material was not reduced so that the immunoglobulin disulphide bonds are intact. *B*, Material was treated with 2-mercaptoethanol to reduce disulphide bonds.

κ constant regions are nearly the same as the corresponding murine amino acid sequences¹⁵; therefore the difference in mobility does not reflect simply a difference in molecular weight. Work is in progress to determine the reason for these differences in mobility.

We used two methods to compare the binding sites of mouse and chimaeric IgM. First, we determined the affinity of these IgMs for TNP by measuring the ability of free hapten to inhibit the inactivation by these IgMs of TNP-coupled phage T4 (Fig. 3); the association constant of mouse and chimaeric IgM for the hapten TNP-cap (2,4,6-trinitrophenyl- ϵ -aminocaproic acid) was $1.3 \pm 0.5 \times 10^4 \text{ M}^{-1}$ and $1.5 \pm 0.5 \times 10^4 \text{ M}^{-1}$, respectively. Within experimental error, we can detect no significant difference in affinity for TNP between the mouse and chimaeric IgMs.

We also compared the affinity of these IgMs for each of several trinitrophenyl-like compounds by measuring the ability of these compounds to block the agglutination of TNP-SRBC by IgM. Figure 4 illustrates the results obtained for TNP-cap and DNPy-Ala (3,5-dinitropyridine- β -alanine). The displacement of the inhibition curves indicates that the affinity of each IgM for DNPy-Ala is about threefold less than for TNP-cap. Table 1 summarizes the results obtained for other compounds. Again, these results suggest that the hapten binding sites are comparable in the mouse and chimaeric IgMs. The direct analysis of immunoglobulin structure predicted that immunoglobulin specificity should be unchanged when the same variable region is joined to different constant regions¹⁶. This prediction has been verified both in studies of IgM and IgD in normal cells¹⁷ and in analyses of the binding specificity of the immunoglobulin made by hybridoma cell lines which have switched *in vitro*^{18,19}. In addition, Sharon *et al.*²⁰ have shown that the substitution of a light-chain constant region for a heavy-chain constant region does not affect the affinity of the resulting protein. On the other hand, we can distinguish between the chimaeric and mouse IgMs using hapten inhibition of TNP-SRBC agglutination as a binding assay (Fig. 4). For each hapten, the curve for chimaeric IgM is displaced from the corresponding curve for mouse IgM. The phage inactivation analysis described above indicated that

Table 1 Relative affinity of mouse and chimaeric IgMs for trinitrophenyl-like compounds

Hapten	mIgM	χ -IgM
TNP-cap	1.00	1.00
TNP-Lys	1.00	1.00
DNP-Lys	1.00	1.00
DNP γ -Ala	3.00	3.00
NIP	>10	>25
NP	>10	>25

As described in Fig. 4 legend, we measured the ability of each hapten to inhibit agglutination of TNP-SRBC. TNP-cap was obtained from Dr G. D'Agostura; TNP-Lys (2,4,6-trinitrophenyl-L-lysine) and DNP γ -Ala were obtained from Research Plus; NIP (4-hydroxy-3-iodo-5-nitrophenylacetic acid) and NP (4-hydroxy-3-nitrophenylacetic acid) were obtained from Sigma and Aldrich, respectively. The affinity of each IgM for the indicated haptens, relative to TNP-cap, was determined by the displacement of the inhibition curves. mIgM, murine IgM; χ -IgM, chimaeric IgM.

these IgMs have the same affinity for TNP-cap. In this context, the displacement of the agglutination curves suggests that the binding of the chimaeric IgM to TNP-SRBC is different in some way from that of the mouse IgM. The significance of this difference in molecular terms is unclear. Crystallographic analysis has suggested that there are several sites at which the variable region interacts with the first domain of the constant region¹⁶; such interactions may affect the binding site differently in the chimaeric and mouse IgMs. On the other hand, agglutination is a complex process involving the binding of multiple IgMs at each of the multiple sites. Subtle differences in aspects such as flexibility of the constant region may affect binding and thus the sensitivity to inhibition by free hapten. The molecular stress necessary to effect agglutination may distort the variable region in ways which would not usually occur while the immunoglobulin is free in solution.

We have also compared the chimaeric and mouse IgMs for their ability to activate complement. Culture supernatants were titred for their ability to promote lysis of TNP-SRBC in the presence of a source of complement, that is, guinea pig serum. Compared with the haemagglutination titre, the haemolysis titre for chimaeric IgM was about fourfold less than that for mouse IgM; we do not know whether this reflects a difference in the intrinsic ability of these IgMs to activate complement or the difference in TNP-SRBC binding discussed above.

Several therapeutic uses for specific antibodies have been proposed. The transfer of passive immunity by injecting specific immunoglobulins is a long-standing treatment. Other uses are more speculative. In animal models, anti-idiotypic antibodies have been used both to elicit and to suppress the production of specific antibodies by the recipient animal²¹. These results might be extended to humans so that anti-idiotypic antibodies could be used in some cases as a vaccine to enhance antibody production and in other cases to depress the destructive immune responses which cause autoimmune diseases. The identification of tumour-associated antigens might lead to the production of monoclonal antibodies that selectively destroy tumour cells *in vivo*²². However, as mentioned above, it may prove difficult to obtain human monoclonal antibodies having the specificities required for immunotherapy. Chimaeric immunoglobulins may provide a good compromise. For both monoclonal human immunoglobulin and chimaeric immunoglobulin, the constant region is expected to be non-immunogenic. We have no reason to expect the mouse variable region would in this form be more immunogenic than a human variable region of the same specificity.

In terms of DNA and protein, the chimaeric antibody system works well. The regulatory signals for RNA transcription, initiation, termination and splicing function, so that these genes express a high level of chimaeric μ and κ chains. The specificity of the mouse variable region is preserved in the chimaeric IgM, and the human constant regions function so that the μ and κ

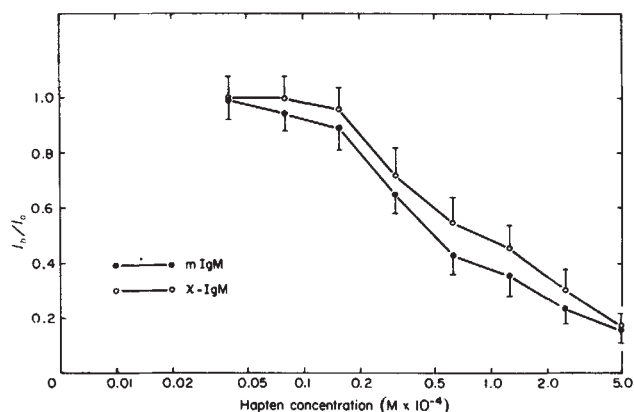


Fig. 3 Affinity of chimaeric and mouse IgMs for TNP. TNP was coupled to phage T4 as described elsewhere²⁴. The affinity for hapten was calculated as follows. The rate-limiting step for the inactivation of phage T4 is the attachment of the first immunoglobulin binding site²⁵, that is, the concentration of surviving phage, Φ , is given by $\Phi = P \exp(-aB)$ where P = initial haptenated phage concentration before reacting with the anti-TNP, B = concentration of free binding sites and a = proportionality constant. By incubating free hapten in the reaction mix, the concentration of free binding sites can be manipulated according to the formula $K_a = [hB]/[h][B]$, where K_a is the association constant, $[h]$ the hapten concentration, $[B]$ the concentration of free binding sites and $[hB]$ the concentration of binding sites bound to hapten. The inactivation index, I_h , is defined as $\log \Phi(h)/P$ where $\Phi(h)$ is the phage concentration after incubation with anti-TNP in the presence of hapten at concentration h . The figure plots the ratio I_h/I_0 as a function of hapten concentration where I_0 is the inactivation index obtained in the absence of free hapten ($h=0$). Each point was determined in triplicate. The affinity constant for the hapten can be calculated from any point as $K_a = 1/h[I_0/I_h - 1]$. The values of K_a given in the text represent the average of all points.

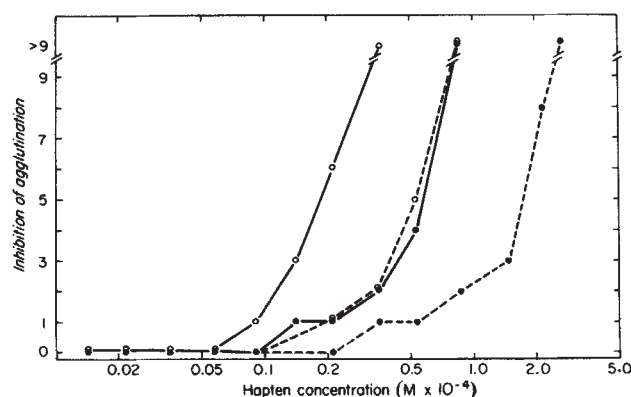


Fig. 4 Relative affinity of mIgM (●) and χ -IgM (○) for TNP (—) and DNP γ (---) haptens. Culture supernatants from the transformants TSp2/mIgM12 and TSp2/ χ -IgM1 were serially diluted in twofold steps and incubated with either TNP-cap or DNP γ -Ala at the indicated concentration in V-bottomed 96-well trays. TNP-SRBC were then added and the wells were scored for agglutination. The inhibition of agglutination, that is, the reduction in the number of wells with positive haemagglutination, is shown here for the indicated concentrations of each hapten. The displacement of the curves for the different haptens indicates the hapten concentrations required to yield the equivalent number of free binding sites, and thus measures the relative affinities of each IgM for the two haptens²⁶.

chains are covalently bound to form pentameric IgM which can activate complement. We have, nevertheless, detected differences in the binding to TNP-SRBC of the chimaeric and mouse IgMs. Further work is needed to assess whether these differences have significance for immunotherapy.

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Participation of p53 cellular tumour antigen in transformation of normal embryonic cells

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The cellular tumour antigen p53 is found at elevated levels in a wide variety of transformed cells (for reviews see refs 1, 2). Very little is yet known about the precise relationship of p53 to malignant transformation. Although the increase in p53 levels could be a secondary by-product of the transformed state, it is equally possible that p53 is actively involved in altering cellular growth properties, especially as it has been implicated in the regulation of normal cell proliferation³⁻⁶. We sought to test whether p53 could behave in a manner similar to known genes in a biological test system, and we demonstrate here that p53 can cooperate with the activated Ha-ras oncogene to transform normal embryonic cells. The resultant foci contain cells of a markedly altered morphology which produce high levels of p53. Cell lines established from such foci elicit tumours in syngeneic animals.

Recent findings have suggested certain similarities between p53 and the product of the oncogene *myc*. Both are DNA binding proteins (ref. 7 and D. Lane, personal communication) that accumulate in the nuclei of transformed cells^{7,8}. Both are regulated with the cell cycle^{9,10} and are induced at an early stage following the treatment of resting cells with mitogens^{3,5,9,10}. Cycloheximide-treated cells accumulate p53¹¹ and show increased *myc* messenger RNA levels⁹. Detailed analysis of the amino acid sequences predicted for the two proteins shows weak similarities in both the overall molecular organization and the positioning of charged residues within distinct domains of the molecules^{12,13}. Hence, if p53 can function like known oncogenes, it is likely to do so in a manner similar to *myc*.

Table 1 Transformation of rat and Chinese hamster embryo fibroblasts by various gene combinations

Transfected DNA	Foci per 10 ⁶ cells			Tumorigenicity
	REF expt 1	REF expt 2	CHEF expt 3	
Carrier (BALB/c DNA)	0	0	0	
pEJ6.6	0	0	0	
pMSVp53G	0	0	ND	
pPyp53c	ND	ND	0	
pMSVp53G + pEJ6.6	13	5	ND	15/15
pPyp53c + pEJ6.6	ND	ND	20	
pLSVmyc + pEJ6.6	ND	21	ND	9/9
pLA8 + pEJ6.6	68*	ND	165*	
pMSVE + pEJ6.6	ND	0	ND	
None				0/8

Primary Fisher rat or Chinese hamster embryo fibroblasts were prepared³⁰ and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 4 mM L-glutamine (maintenance medium). 10⁶ cells were seeded per 90-mm dish, and were transfected with the indicated DNA combination after 1 day (10 µg of each plasmid, made up to a total of 25 µg with sheared BALB/c liver DNA). pLSVmyc was constructed linking the 5.6 kb *Bam*HI fragment of the murine *c-myc* gene (see ref. 14) to the SV40 early promoter. pLA8 contains the left-end 9.1% of the adenovirus12 genome, including the E1A and part of the E1B region¹⁶. pMSVE is a derivative of pMSVp53G, containing only the MSV enhancer inserted in the *Bam*HI site but no p53-specific sequences (compare with Fig. 1). The cells were transfected by the calcium phosphate procedure³¹ for 16 h, glycerol-shocked (10% glycerol in maintenance medium for 90 s), replenished with maintenance medium, allowed to recover for an additional day, then split and reseeded at a density of 4 × 10⁵ (rat fibroblasts) or 2 × 10⁵ (Chinese hamster cells) per 90-mm dish. Medium was changed every 6-7 days. Distinct foci overgrowing the monolayer were scored 16 days (expt 1), 14 days (expt 2) or 24 days (expt 3) after replating of transfected cultures. REF, rat embryo fibroblasts; CHEF, Chinese hamster embryo fibroblasts. The tumorigenicity of cell lines established from corresponding foci was determined by injecting subcutaneously 5 × 10⁶ cells into 5-8 day-old Fisher rats whole-body irradiated with 200 rads. As a non-transfected control (bottom line) we used REF propagated in culture to obtain a sufficient number of cells. Data are derived from two different p53 + Ha-ras lines and one *myc* + Ha-ras line. ND, not determined.

* Foci possessing a distinctly different morphology from that induced by pLA8 alone.

A biological test system demonstrating the involvement of the *myc* product in malignant transformation has been established recently^{14,15}; primary rat embryo fibroblasts are transformed stably by the joint action of *myc* and another oncogene such as Ha-ras. Similar results are obtained when another nuclear oncogene, the adenovirus-2 E1A region, is assayed by co-transfection with Ha-ras in an analogous system¹⁶. In both cases, the transformation is visualized by the appearance of dense foci capable of overgrowing the monolayer of normal cells and are dependent on the presence of both oncogenes. This system is therefore a suitable test of the oncogenic properties of p53.

Two types of recombinant DNA constructs were used as templates for efficient p53 expression in transfected cells (Fig. 1). The plasmid pMSVp53G contains the 16 kilobase (kb) *Eco*RI fragment encompassing the functional murine p53 gene^{12,17} juxtaposed to the enhancer portion of the Moloney murine sarcoma virus (MoMSV) long terminal repeat. This approach utilizes the presence of a functional promoter in the 16 kb fragment (B. Bienz, unpublished results). The second construct, pPyp53c, contains a stretch of p53 cDNA linked to the polyoma virus early promoter; this cDNA contains the intact coding region for p53¹⁷ and directs the synthesis of authentic p53 in a heterologous system¹⁸.

Secondary Fisher rat embryo fibroblasts were co-transfected with pMSVp53G and pEJ6.6 (ref. 19), carrying an activated human *c-Ha-ras*1 gene. As a positive control, we performed a

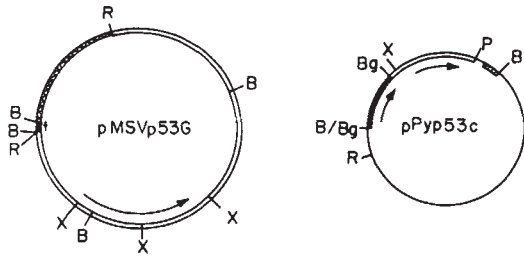


Fig. 1 p53-specific plasmids used for transfection. pMSVp53G contains the functional mouse p53 gene linked to the enhancer element of MoMSV DNA. The 16 kb *EcoRI* fragment was excised out of the recombinant phage Ch 53-7 (ref. 12) and inserted into the *EcoRI* site of plasmid pA₁₀ (ref. 32). The *HinI*-*XbaI* fragment of the MoMSV long terminal repeat, containing the enhancer portion³³, was converted at both ends to *Bam*HI and introduced into the *Bam*HI site of the same pA₁₀ plasmid. Hatched bar, pA₁₀ DNA; full bar, MoMSV DNA; open bar, p53-specific genomic DNA. To construct pPyp53c, the *Bam*HI-*Hph*I fragment of polyoma (Py) DNA, containing the early promoter³⁴, was converted to *Bgl*II at both ends. This was inserted into pSVp53c17 DNA¹⁸, cleaved previously with *Bgl*II and partially cleaved with *Bam*HI to remove the bulk of SV40 sequences. Briefly, pPyp53c consists of the following components (from 5' to 3') inserted within the *Bam*HI site of pBR322: first, the Py early promoter region, a segment of p53 cDNA extending from the *Ban*I site at nucleotide -67 (ref. 17) to the end of the insert of pp53-176 (nucleotide 1,188, ref. 17) and including also 25 base pairs of oligo(dG):oligo(dC); second, 125 base pairs of pBR322 DNA corresponding to the region between the *Pst*I site and the nearby *Bgl*II site³⁵; finally, the *Bcl*I-*Bam*HI fragment of SV40 DNA, derived from plasmid pLSV and including the viral early polyadenylation site³⁶. Thin line, pBR322 sequences; full bar, Py DNA; open bar, p53 cDNA; hatched bar, SV40 DNA, B, *Bam*HI; Bg, *Bgl*II; P, *Pst*I; R, *Eco*RI; X, *Xba*I. For clarity, only the *Pst*I site originally harbouring the cDNA insert of clone pp53-176 (ref. 17) is indicated. Arrows, normal transcriptional orientation.

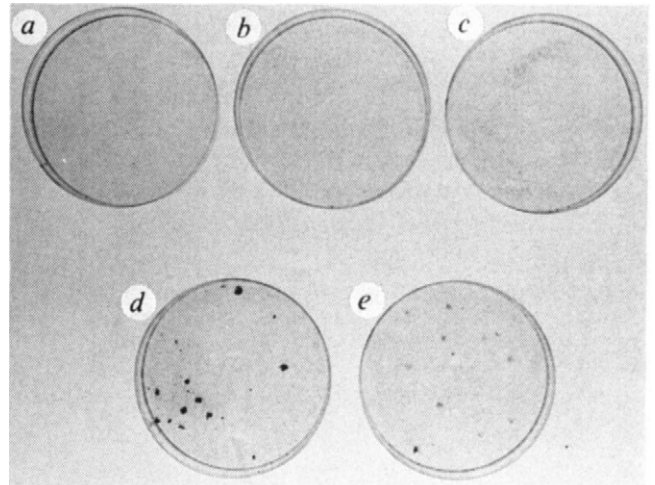


Fig. 2 Giemsa-stained rat embryo fibroblast cultures transfected with various DNA combinations. Transfection was as described in Table 1, using the following DNA: a, BALB/c carrier DNA alone; b, pEJ6.6+carrier; c, pMSVp53G+carrier; d, pLA8+pEJ6.6 carrier; e, pMSVp53G+pEJ6.6+carrier. Photographs were taken 16 days after replating of transfected cells.

co-transfection with pEJ6.6 and pLSVmyc. The latter plasmid contains the protein-coding part of the mouse *myc* gene, driven by the simian virus 40 (SV40) early promoter (see Table 1). In some cases, pLSVmyc was replaced by pLA8 (ref. 16), containing the left-end 9.1% of the adenovirus-2 genome.

We found that although neither p53 nor Ha-ras alone induced the appearance of foci, their joint action could cause this effect (Table 1). The foci generated had a distinct morphology (Fig. 2) and could usually be detected after 9–12 days. In these experiments, p53 was less efficient than *myc* and markedly less efficient than pLA8. Although pLA8 by itself is sufficient for transformation¹⁶, the foci induced by its co-transfection with pEJ6.6 grew much more rapidly than those due to pLA8 alone and had a very different, easily recognizable morphology (data not shown).

To test that the observed co-transformation is not due to activation of unrelated genes by the enhancer in pMSVp53G^{20,21}, the latter plasmid was substituted by an analogue lacking p53 sequences; no foci were generated (Table 1).

The morphology of the foci cells varied with the particular gene combination, although all were clearly distinct from the surrounding non-transformed cells. Cells transformed by p53+Ha-ras (Fig. 3g) were large, refractile and more similar to the *myc*+Ha-ras transformants (Fig. 3e), whereas the foci arising in monolayers transfected with pLA8+Ha-ras were composed mostly of smaller cells (Fig. 3c). p53+Ha-ras foci tended to grow more slowly than *myc*+Ha-ras foci and often appeared to stop spreading after reaching 5–7 mm diameter, whereas *myc*+Ha-ras foci grew continuously until they merged and filled the dish.

Similar results were obtained using an alternative system, secondary Chinese hamster embryo fibroblasts and the cDNA construct pPyp53c (Table 1). Co-transfection with p53 and pEJ6.6 resulted in overgrowing foci of cells with transformed

morphology (Fig. 3h,i). However, these experiments were hampered because the transformed cells were prone to lysis.

If p53 production is involved in the observed transformation, the protein should be made in the transformed cells. Accordingly, cells were isolated from foci, labelled with ³⁵S-methionine and assayed for p53. We used anti-p53 monoclonal antibody RA3-2C2 (refs 22, 23); this is specific for the murine form of the protein and does not cross-react with rat p53 (ref. 24), thus allowing the detection of transfection-derived murine p53 in the transformed rat cells and avoiding potential difficulties due to the presence of endogenous rat p53. Murine p53 could be immunoprecipitated easily from overgrowing foci cells (Fig. 4), sometimes in even higher concentrations than those present in Meth A mouse fibrosarcoma cells, marked overproducers of p53 (ref. 25). This suggests strongly that the actual production of the protein is involved in the transformation.

Unlike *myc*+Ha-ras or pLA8+Ha-ras, it proved difficult to establish cell lines from foci induced by p53+Ha-ras. Typical transformed cells exhibited a limited proliferation potential and, after repeated passaging, cultures became dominated by cells of a normal-nontransformed morphology, probably derived from the underlying monolayer. The level of murine p53 fell simultaneously to below detection after two or three passages (data not shown). These findings support the notion that p53 production, directed by the transfected DNA, is involved in the observed transformation. Almost all cells of distinct transformed morphology, when transferred into a new dish, attached to it but did not divide. On the other hand, the few normal cells transferred with the focus did start dividing once replated at a low density. Eventually, with several low-density passages, replicating normal cells became predominant. These data suggest that, unlike *myc* or EIA^{26,27}, p53 is not capable of efficiently immortalizing cells. Thus, the p53+Ha-ras transfectants, although transformed, still undergo a senescence crisis and stop multiplying. The normal underlying cells, however, are still able to resume replication once subcultured at a low density, hence they can easily overgrow the senescent transformed cells. Nevertheless, a very small proportion of transformed cells did eventually give rise to stable transformed cell lines. Thus, when foci were subcultured and cells maintained in the dish for 10–14 days without further passaging, a few overgrowing foci became evident. The cells comprising these secondary foci, while generally morphologically indistinguishable from those found in the original foci, did possess an apparently unlimited growth

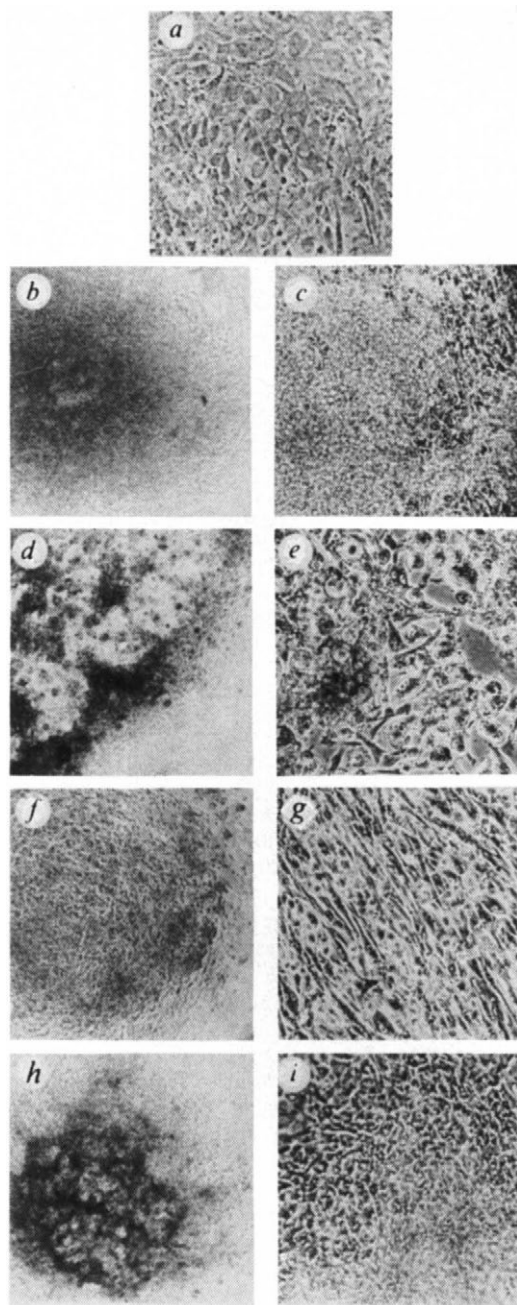


Fig. 3 Morphology of cells transformed by various plasmid combinations. Pictures were taken at two magnifications to display the typical appearance both of the foci and of individual cells within them. *a*, REF (Table 1) transfected with carrier only; *b, c*, REF transfected with pLA8+pEJ6.6; *d, e*, REF transfected with pLSVmyc+pEJ6.6; *f, g*, REF transfected with pMSVp53G+pEJ6.6; *h, i*, CHEF transfected with pPyp53c+pEJ6.6. Normal CHEF are visible in the upper left corner of *i*. Magnification: *b, d, f, h*, $\times 40$; *a, c, e, g, i*, $\times 100$.

potential and could be propagated easily to yield homogeneous stably-transformed cell lines. When two such lines were subjected to more detailed analysis, both expressed high levels of murine p53 (data not shown). On injection into syngeneic young rats, both cell lines generated tumours at a high efficiency (Table 1) which developed significantly more slowly than those induced by pLSVmyc + EJ6.6 transformants. In some animals, they stopped growing after reaching 6–10 mm diameter, whereas in most cases they grew continuously, reaching a very large size and eventually killing the animal. We are currently testing more lines

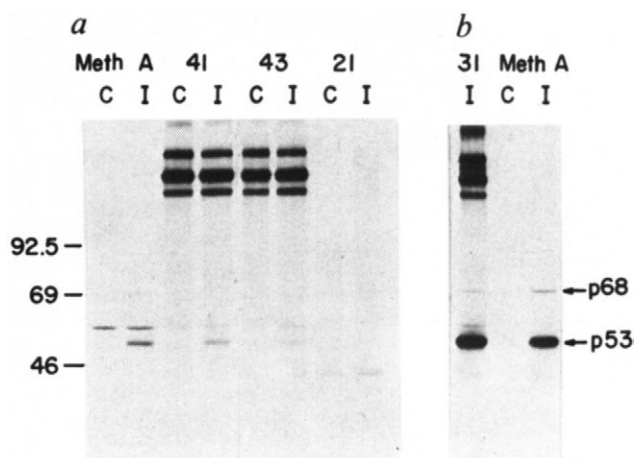


Fig. 4 Analysis of proteins produced in focus-derived rat cells. Foci arising in a monolayer of rat embryo fibroblasts following transfection with different DNA combinations were trypsinized, expanded into 35-mm Petri dish cultures and labelled with $40 \mu\text{Ci}$ ^{35}S -methionine for 4 h. Extracts were prepared as described previously³⁷. Equal amounts of trichloroacetic acid-insoluble radioactivity (2.8×10^6 c.p.m. per lane) were immunoprecipitated with either anti-mouse p53 monoclonal antibody RA3-2C2^{22–24} or control serum, using standard procedures³⁸, followed by electrophoresis through a 12.5% SDS-polyacrylamide gel³⁸. *a, b* refer to two separate experiments. *a*, Autoradiography for 2 days; *b*, for 7 days. Meth A are chemically transformed mouse fibroblasts which overproduce p53 (ref. 25); 41, 43 and 31 are derived from foci induced by pMSVp53G+pEJ6.6 while 21 is derived from a focus induced by pLA8+pEJ6.6. C, control serum, I, RA3-2C2 monoclonal antibody. Numbers on the left refer to the relative molecular mass ($\times 10^{-3}$) of co-electrophoresed markers. p68 is a polypeptide co-precipitating with mouse p53 (see ref. 18).

to determine whether this is a general feature of p53+Ha-ras transformants.

Based on the results presented here and in ref. 28, we conclude that p53 can act as a nuclear oncogene by collaborating with Ha-ras in the transformation of cultured embryonic fibroblasts. Unlike *myc* or EIA, only a small proportion of the resultant transformants eventually become immortalized and give rise to stable lines, possibly through a change involving a third gene product in addition to p53 and Ha-ras. The initial transformed cells must be highly predisposed towards this activation event, as it occurs much more frequently than predicted for random mutations. Alternatively, the few immortalized cells may be selected for their ability to produce increased amounts of either p53 or Ha-ras protein. Recent data²⁹ suggest that massive overproduction of the Ha-ras p21 protein can circumvent the need for a cooperating oncogene. The rare alteration leading to immortalization may thus be quantitative rather than qualitative. Immortalization may not be a general attribute of nuclear transforming proteins. Although *myc* and EIA can efficiently immortalize cells^{26,27}, their ability to induce overgrowing foci in combination with Ha-ras may reflect an entirely different facet of their activity, the two oncogenes sharing only certain properties with p53.

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Cooperation between gene encoding p53 tumour antigen and *ras* in cellular transformation

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The protein p53 is highly expressed in a large variety of transformed cell types originating from diverse species. These include cells transformed by Simian virus 40 (SV40), adenovirus and Abelson virus, as well as a variety of chemically transformed cells¹⁻⁶. Substantial amounts of p53 are also present in certain non-transformed cells, for example, some embryonic tissues⁷. The protein may be localized in different cellular compartments in normal and transformed cells⁸. The strong correlation between tumorigenicity and high levels of p53 suggests an important role of p53 in tumorigenesis. We report here experiments in which we have co-transfected the murine cellular gene encoding for p53 with a *ras* gene into primary rat embryo fibroblasts. Our results indicate that the p53-encoding gene can play a causal role in the conversion of normal fibroblasts into tumorigenic cells.

A cDNA clone of the p53 gene^{9,10} was used to retrieve a homologous 16 kilobase *EcoRI* segment from a genomic library made from DNA of a murine B-cell lymphoma induced by Abelson virus¹¹. This genomic clone was modified by linkage to the murine leukemia virus (MLV) promoter-enhancer long terminal repeat element. These long-terminal repeat sequences were inserted to induce a high level of expression of the cloned p53 gene (Fig. 1).

We performed transfection experiments with clones of the modified genes (see Fig. 1) to determine whether these clones

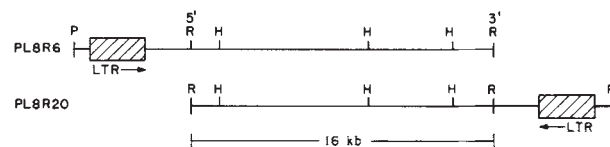


Fig. 1 Schematic diagram of p53 encoding plasmids. (For detailed description, see ref. 15.) Briefly, the 16kb mouse DNA fragment containing all of the p53-encoding exons was inserted either downstream from and in the same transcriptional orientation as the MuLV long terminal repeat (PL8R6), or upstream and in the opposite transcriptional orientation (PL8R20). The vector is pBR322. R, *EcoRI* restriction sites; H, *HindIII* restriction sites; P, *PstI* restriction sites.

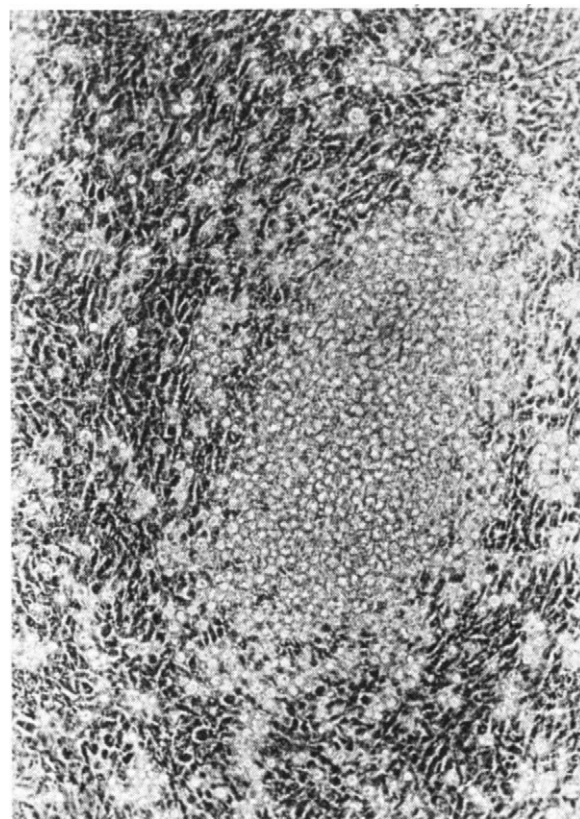


Fig. 2 Phase contrast photomicrograph of a focus arising in a dense monolayer of normal rat embryo fibroblasts after co-transfection of PL8R20 and the EJ-*ras* oncogene. The DNA transfection was performed as described in Table 1. The photograph was taken 10 days after transfection.

would induce foci in monolayer cultures of the Rat-1 fibroblast cell line or in cultures of secondary rat embryo fibroblasts (REFs). All transfections include the pSV2neo³³ plasmid DNA so that a portion of the cultures could be placed under G418 drug selection to control for transfection efficiencies.

The various p53 clones tested are unable to induce foci in any of the transfected Rat-1 cultures, even when such cultures are surveyed weeks after transfection (Table 1). In the same experiments, a *ras* oncogene elicits foci observable in Rat-1 cells within eight days of transfection. We conclude that the p53 gene, unlike the *ras* oncogenes, has no apparent ability to transform readily established rodent fibroblast cells.

Earlier work had shown that a single oncogene, such as *ras* or *myc*, is unable to induce foci in REF monolayer cultures. However, when the two oncogenes are applied together, foci are induced which contain cells that are tumorigenic in young rats or nude mice^{12,13}. The observed inability of *ras* or p53 clones to yield foci in transfected REF cultures (Table 1) is consistent with these earlier results.

Table 1 Transforming of REFs and Rat-1 cells after co-transfection of the p53-encoding gene and the EJ-c-Ha-ras oncogene

Transfected gene	No of foci per 10 ⁵ cells		Tumorigenicity of REFs nude mice (No. of tumours/no. of injections)
	REFs	RAT-1	
REF/DNA	0	0	0/10
pEJ6.6	0	2,000–2,500	0/20
psvcmv-1	0	0	0/15
pEJ6.6 + pSVcmv-1	200–300	2,000–2,500	†29/29
pL8R6	0	0	0/5
pL8R20	0	0	ND
pL8R6 + pEJ6.6	20–30	2,000–2,500	‡6/6
pL8R20 + pEJ6.6	10–20	2,000–2,500	§3/3
pL8R6 + pSVcmv-1	0	0	0/6
pL8R20 + pSVcmv-1	0	0	ND
*pL8R6 (<i>Bam</i> HI/ <i>Hind</i> III + pEJ6.6)	0	ND	0/3

The preparation of primary cultures of Fisher REFs the transfection procedures and plasmids pEJ6.6 and pSVc-myc1 are described elsewhere¹². 0.5 µg of PSV2neo³³ DNA per 2 × 10⁶ cells was included in all transfections. Subcutaneous injections to test for tumorigenicity made into 30–40-day-old nude mice irradiated (500 rad) 24 h before. All injected mice were observed for five weeks. ND, not determined.

* PL8R6 digested to completion with restriction endonucleases *Bam*HI and *Hind*III to inactivate the *p53* gene but not the MLV-LTR promoter-enhancer. At 6 weeks, one of these mice exhibited a small tumour. The nature of this tumour is under study.

† Tumours detected 10–14 days after injection.

‡ Tumours detected 15–20 days after injection.

§ Tumours detected 20–25 days after injection.

The previously observed cooperation of *ras* and *myc* oncogenes suggests an experimental test by which the oncogenic function of other genes can be assayed: a DNA clone carrying a gene of interest is co-transfected with either a *ras* or a *myc* clone and the appearance of REF foci then scored. Accordingly, we tested the two *p53* gene clones for cooperation with *ras* or *myc* oncogenes in the transformation of REFs. Neither of the two *p53* constructions induce foci when co-transfected with a *myc* oncogene clone. However, when these *p53* constructions were tested in conjunction with the human EJ/T24 Ha-*ras* oncogene, they do cooperate in focus induction. These *ras-p53* foci appear with the same kinetics as *ras-myc* foci, but with 10-fold lower frequency. The *ras-p53* foci are quite large, containing very refractile and rounded cells which do not adhere well to the culture dish (Fig. 2).

We next attempted to demonstrate that the entire *p53* chimeric gene, and not simply attached promoter-enhancer sequences, cooperate with the *ras* gene in transformation. The *p53* clone pL8R6, which is active in the co-transfection assay, was digested to completion with the restriction endonucleases *Bam*HI and *Hind*III. These enzymes cut the *p53* domain into several fragments but leave the linked long-terminal repeat promoter-enhancer segment intact. Co-transfections of the digested clone and the *ras* oncogene do not yield any foci in REFs. We conclude that the sequences within the *p53* gene itself are required to cooperate with *ras* in transforming REFs *in vitro*. A second type of promoter-enhancer, introduced at the 3' end of the *p53* gene, is unable to adequately activate the gene (data not shown). This and other evidence (ref. 11; D.W. & V.R., unpublished observations), suggests that appropriate transcriptional activation of the *p53* clone is required for biological activity.

A more stringent test for transformation than that of focus induction is the assay for tumorigenicity. Fourteen days after transfection, we injected nude mice with the cells from transfected cultures. Only a small proportion of cells from such cultures have acquired and expressed the transfected oncogenes; such transfected cells have not had time to undergo extensive additional alterations, such as may occur during isolation of clonal lines before injection. These injected cultures include the doubly transfected cultures *ras + myc* or *ras + p53*, as well as those singly transfected with either *ras* or *myc* or *p53* clones (Table 1). Ten days after injection, tumours appear at all the sites injected with *ras + myc* transfectants, whereas only small nodules are visible at the sites carrying *ras + p53* cells. After 18 days, however, all sites injected with *ras + p53* transfectants exhibit large, aggressively growing tumours.

Cultures carrying *myc + p53* co-transfected cells develop no foci and yield no tumours upon injection. Thus it appears that *p53* is able to function analogously to *myc* by cooperating with

ras oncogenes to induce tumorigenic conversion of rat embryo fibroblasts. The correlation was absolute between the ability of co-transfected cloned DNAs to induce foci in REF monolayers and the ability of these DNAs to yield tumorigenic cultures.

The delayed appearance of *ras + p53* tumours may be due to the smaller initial number of transformed cells present in the inocula. *Ras + myc* transfected dishes contain on average 50 foci. Consequently, each inoculum of 10⁶ cells contains about 2.5 × 10⁴ transformed cells. *Ras + p53* dishes contain approximately five foci and only about 2,500 transformed cells present among the normal rat embryo fibroblasts. Although delayed in appearance, the tumours stemming from these *ras + p53* transfectants rapidly reach the size of *ras + myc* tumours. All tumorigenic lines resulting from *ras + p53* co-transfection carried intact, acquired *p53* DNA segments (data not shown).

The existence of a *p53*-specific monoclonal antibody, RA3-2C2^{14,15}, facilitates the protein analysis of the *p53* transfectants. This antibody is specifically reactive only with mouse *p53*¹⁵. The *p53* DNA clones used here were isolated from DNA of the Abelson virus-transformed, mouse lymphoid line 230–23–8 (ref. 11). This antibody allows the specific detection of *p53* expressed by introduced DNA clones because it has no cross-reactivity with the endogenous, homologous *p53* protein of the rat host cell. All transfectants demonstrate the presence of a mouse *p53* band, although at differing levels (Fig. 3). This suggests that the continued presence of *p53* is required to maintain the transformed phenotype.

The results presented here and in the accompanying paper of Eliyahu *et al.*¹⁶, demonstrate that a modified *p53* gene can function as an active oncogene that contributes to tumorigenic conversion. We conclude that expression of *p53* can be more than a consequence of the cancer state. Instead, it appears that an active *p53* gene can play a causal role in tumorigenicity and can be classified as an oncogene. The *p53* oncogenic functions are analogous to cellular *myc* and *myb* oncogenes. (H.L. *et al.*, manuscript in preparation). Although weaker than *myc* in its ability to cooperate with *ras*, we predict that the two genes, *p53* and *myc*, will be shown eventually to express many similar functions.

In addition to the functional analogies between *p53* and *myc*, some biochemical similarities are apparent. Both genes encode nuclear proteins^{17–19}. In nontransformed cells, these proteins are metabolically highly unstable, with half lives of less than 60 min (refs 20, 21; R. Eisenmann, personal communication). Both the proteins are phosphorylated^{22–24} and their expression is linked strongly to the growth state of the cell. In both cases, large increases in expression can be observed following serum stimulation of quiescent cells^{25,26}. This increase of *p53* expression seems to be required for induction of DNA replica-

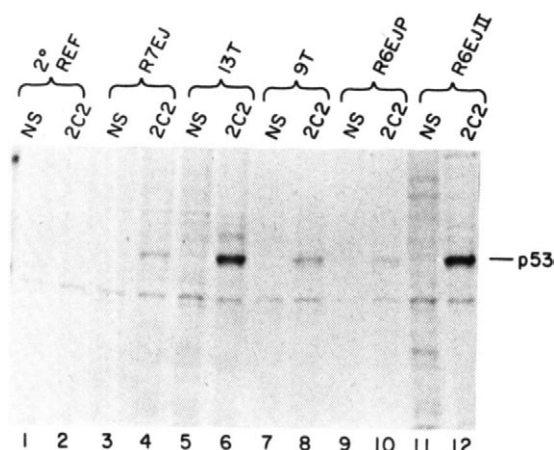


Fig. 3 Analysis of p53 protein cell lines and tumours derived from rat embryo fibroblasts co-transfected with the p53 gene and the EJ-ras oncogene. Cells in 60 mm dishes were labelled for 2 h with 100 μ Ci [35 S]-methionine in dialysed calf serum and methionine-free Dulbecco's Modified Eagles medium. Lysates were prepared as described previously¹⁵ and incubated for 1 h at 4°C either with normal rat serum (lanes 1, 3, 5, 7, 9, 11) or with monoclonal antibody RA3-2C2^{20,21} (lanes 2, 4, 6, 8, 10, 12). Protein A Sepharose was added and the lysates incubated at 4°C in a rocking bath for 1 h. Pellets were washed 5 times with RIPA buffer, boiled for 3 min and loaded onto a 10% Laemmli gel³², run at 150 V for 5 h. Lanes 1, 2, secondary rat embryo fibroblasts which have not been transfected; lanes 3, 4, R7EJ, a transformed rat embryo fibroblast cell line derived from co-transfection of the EJ-ras oncogene and PL8R7 (vector not described here), a p53 gene with an SV40 promoter; lanes 5, 6, 13T, a cell line prepared from an explanted nude mouse tumour created by injection of the cell line R7EJ; lanes 7, 8, 9T, a cell line prepared from an explanted nude mouse tumour created by injection of the R6EJP cell line; lanes 9, 10, R6EJP, a transformed cell line derived from co-transfection of PL8R6 and the EJ-ras oncogene; lanes 11, 12, R6EJII, an independently isolated transformed rat embryo fibroblast cell line derived from transfection of PL8R6 and the EJ-ras oncogene.

tion. Microinjection experiments have shown that monoclonal antibodies to p53 can inhibit serum-induced DNA synthesis in 3T3 cells^{27,28}.

SV40 and adenovirus both encode proteins that bind to p53^{4,29}. In the case of the SV40 protein, this ability to bind to p53 appears to increase its half life to 24 h^{20,21}. From the results presented here, it seems plausible that part of the physiological effects of the SV40 large T antigen, including its ability to immortalize cells, may depend on its ability to stabilize p53 and thus increase its effective concentration. We surmise that deregulation of p53 expression can be achieved at the transcriptional level as well. By linking the p53 gene to strong promoter-enhancers, we have created a functional state which is analogous to linking of the normal *myc* gene to integrated retrovirus genomes³⁰, or immunoglobulin genes³¹. It remains to be determined whether this transcriptional deregulation is sufficient to create oncogenic activation, or whether additional, undiscovered lesions in the protein-encoding portion of the gene must be present also for an active oncogene to arise.

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Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53

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Malignant transformation of primary cells requires at least two distinct and characteristic alterations in cellular behaviour. The first, cellular immortality^{1,2}, can be induced by chemical carcinogens³ or by cloned oncogenes such as polyoma large T (ref. 4), adenovirus early region 1A (E1A) or the oncogene from avian (MC29) myelocytomatosis virus, *v-myc*. Cells whose *in vitro* lifespan has been extended by these procedures can be fully transformed by transfection with oncogenes belonging to a different complementation group, including genes of the *ras* family, adenovirus Elb and polyoma virus middle T (refs 4, 5). The unstable cellular phosphoprotein p53 is frequently present at elevated levels in transformed cells⁶⁻¹¹ and is stabilized by the formation of complexes with simian virus 40 (SV40) large T or adenovirus Elb 57K protein¹⁰⁻¹³. Although several reports have associated p53 with cell proliferation¹⁴⁻¹⁷, its role remains obscure. We have cloned complementary DNA sequences encoding murine p53¹⁸ and report here that transfection of p53 expression constructs into cells of finite lifespan *in vitro* results in cellular immortality and susceptibility to transformation by a *ras* oncogene.

Adult rat chondrocytes obtained from the xiphisternum undergo ~30 doublings *in vitro* before senescence, with characteristic changes in morphology and cessation of growth. Wistar adult xiphisternum chondrocytes (WAXI) at passage 5 grow slowly, with a doubling time of >60 h. Because spontaneous immortal colonies appear very rarely in such cultures, we have used them in an immortalization assay rather than rat embryo fibroblasts, which we have found to show an irregular and unpredictable pattern of senescence coupled with an unacceptably high incidence of spontaneous immortalization. Furthermore, such cultures transfected with the p53 expression vectors provided a low incidence of immortalization (<2 foci per μ g DNA per 10^6 cells).

We used three p53 expression vectors, the construction and properties of which will be described elsewhere. The standard calcium phosphate co-precipitation method¹⁹ was used to introduce constructs into WAXI cells. In the first round of experi-

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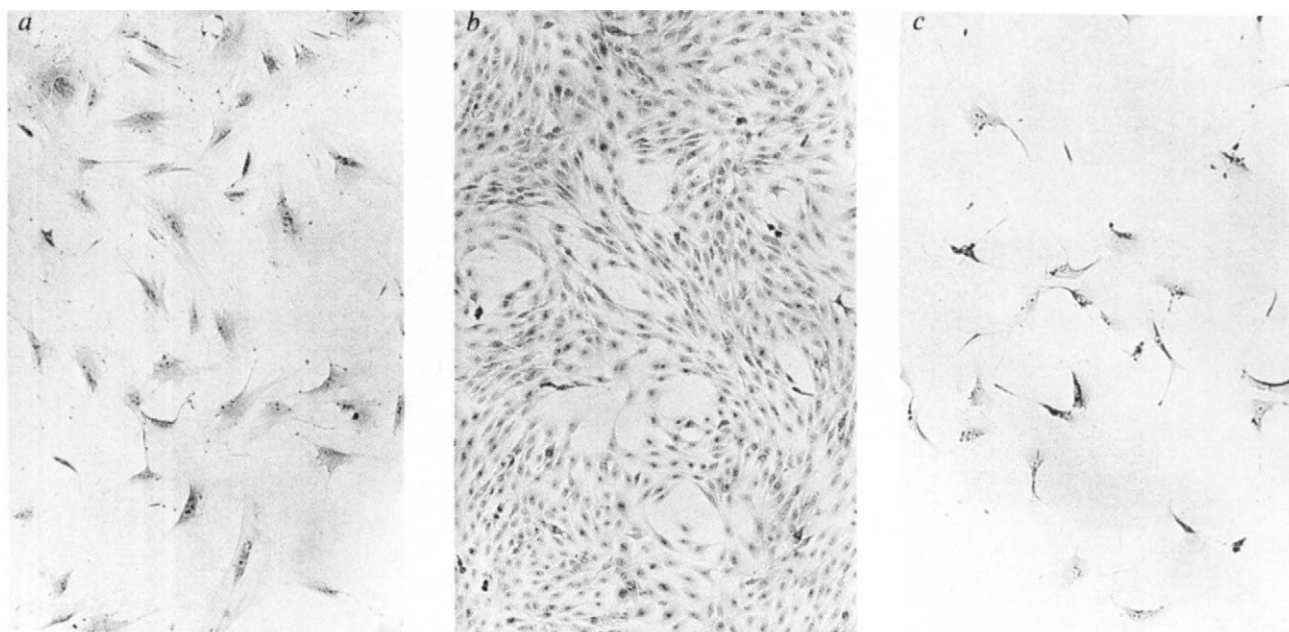
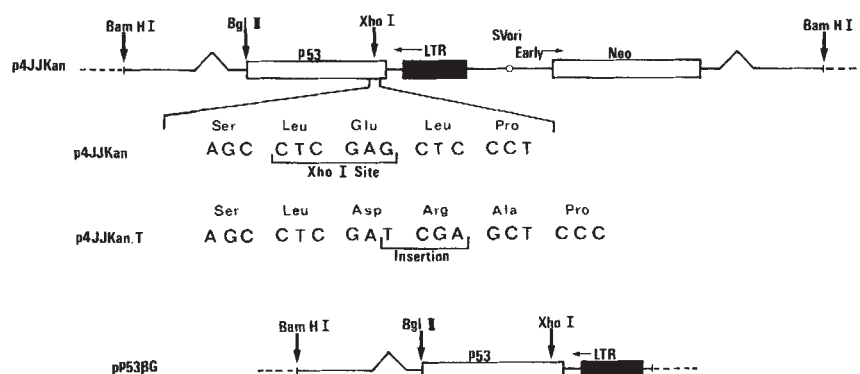


Fig. 1 Adult rat chondrocytes (WAXI). *a*, Calcium phosphate treated; *b*, after transfection with p4JJKan; *c*, after transfection with p4JJKan-T. **Methods:** WAXI cells were derived from the xiphisternum of adult Wistar rats. Fragments of xiphisternum were disaggregated with collagenase and the resulting cell suspension cultured in DMEM containing 10% FCS. When confluent, these cells were passaged using 0.1% trypsin, and low passage stocks were stored in liquid nitrogen. WAXI cells at passage 5 were established in dishes at 2×10^5 per dish in DMEM plus 10% FCS. The following day the cells were subjected to transfection using the standard calcium phosphate method¹⁹, with overnight incubation. Control cultures were treated with calcium phosphate only. Subsequently the cultures were fed thrice weekly and after 7 days were subcultured using a 1:5 split ratio into fresh dishes. Following this passage, untreated cultures regularly senesced. After 21 days culture (during which time cultures transfected with an active p53 expression vector (p4JJKan) had grown rapidly), all the cells were fed with medium containing G418 at $400 \mu\text{g ml}^{-1}$. After 21 days G418 treatment, all control untransfected cultures were dead.

Fig. 2 Schematic diagram of expression constructs containing murine p53 sequences. p4JJKan contains the sequences from pSV2neo (ref. 20) which confer G418 resistance on mammalian cells. Transcription of this gene in eukaryotic cells is directed by the SV40 early promoter. Transcription of p53-5-derived murine p53 cDNA sequences¹⁸ is directed by the Rous Sarcoma Virus long terminal repeat (RSV LTR)^{21,22}. Splicing and polyadenylation signals for this transcript are provided by a second copy of the appropriate sequences from pSV2neo. p4JJKan directs the efficient synthesis of murine p53 protein in transient expression assays using monkey cos-1 cells and mouse specific anti-p53 monoclonal antibodies (data not shown). p4JJKan-T is essentially the same as p4JJKan, but the unique *Xho*I site in the p53 coding sequence has been cut, filled in with T4 polymerase and re-ligated. This generated a frameshift mutation. p4JJKan-T is unable to direct the synthesis of full-length murine p53 protein. This was confirmed in transient expression assays as above. p4JJKan and p4JJKan-T confer kanamycin resistance on their *Escherichia coli* hosts. Transcription of the p53 sequences in pP53 β G is directed by the RSV LTR and splicing signals for this transcript are provided by a *Bal*I-*Bam*HI fragment of the mouse β -major globin genomic gene, which contains the second intron²³. A polyadenylation signal is provided by the 166 base pair *Hpa*I-*Bam*HI fragment of SV40 (nucleotides 2,666-2,533 on the SV40 genome). pP53 β G carries the RTEM-1 β -lactamase gene of pBR322. Open boxes, coding sequence; solid boxes, LTRs; $\wedge$, introns; $\cdots$, flanking pAT153 plasmid sequences.



ments, the constructs were transfected and the cultures were left for 7 days before passaging. They were then split 1:5 and passaged when necessary. On day 21 after transfection, the cultures were treated with the antibiotic G418. By this time, control WAXI cells had undergone senescence and were not proliferating. Cells transfected with the active p53 expression vector p4JJKan continued to proliferate and colonies grew up in $400 \mu\text{g ml}^{-1}$ G418 (see Fig. 1). Cells transfected with p4JJKan-T, which cannot direct the synthesis of full-length p53, provided occasional abortive colonies of senescing cells following G418 selection. Thus, p4JJKan-T conferred G418 resistance on the WAXI cells but failed to extend their lifespan. Immobilization of WAXI cultures by p4JJKan was reproducibly demonstrated in three separate experiments, and was associated with G418 resistance. We also transfected WAXI cells with the

p4JJKan vector and carried out the selection with G418 30 h later. By day 18 after the onset of G418 selection, colonies of proliferating cells were visible in p4JJKan-transfected dishes whereas p4JJKan-T-transfected dishes showed only occasional non-dividing cells (Table 1). We also co-transfected WAXI cells with pP53 β G (Fig. 2) and pSV2neo, followed by G418 selection after 30 h. The results (Table 1) show that plasmid pP53 β G, which uses mouse β -globin splice signals, also gives rise to colonies of proliferating cells by day 18, but pSV2neo does not. Lines of WAXI cells immortalized by p4JJKan and resistant to G418 have been maintained *in vitro* for over 200 doublings. They show no morphological evidence of senescence and continue to double every 18–20 h. Such cell lines are non-tumorigenic when injected into syngeneic or nude rats (5×10^6 cells per site).

Table 1 Phenotypes of rat chondrocytes after transfection with p53 expression vectors and/or T24 Ha-ras1

Plasmid	No. treated	G418-resistant colonies per dish	
Expt <i>a</i>			
None (calcium phosphate)	0/6	0, 0, 0	
p4JJKan	4/4	16, 18, 14	
p4JJKan · T	0/2	0, 0, 0	
pSV2neo	0/4	0, 0, 0*	
Foci per µg DNA per 10 ⁶ cells			
Expt <i>b</i>			
p4JJKan		10, 9	
pP53βG + psV-2-neo		15, 16	
pSV-2-neo		0, 0	
	Anchorage-independent growth (% colony formation)	Doubling time in medium plus FCS at:	
		10%	1%
Expt <i>c</i>			
None (calcium phosphate)	<0.01	>60 h	>60 h
p4JJKan	<0.01	18 h	>60 h
p4JJKan, then pT24-Ha-ras1	27, 32, 35	16 h	16 h
pT24-Ha-ras1	<0.01	ND	ND

Expt a, Rescue of WAXI cells from senescence by transfection with p53 expression vectors. G418 selection was carried out 21 days after transfection. 60-mm dishes were seeded with 5×10^4 cells and cultured in Dulbecco's Modified Eagle's Medium (DMEM) plus 10% fetal bovine serum (FCS). G418 (Geneticin) was used at $400 \mu\text{g ml}^{-1}$. Progressively growing colonies, resistant to G418, were seen only in dishes of WAXI cells previously transfected with the p4JJKan construct. Cells obtained from these colonies have continued to proliferate (>200 doublings) without showing morphological or proliferative evidence of senescence. Expt b, Immobilization of WAXI cells by p53 expression vectors detected as resistant foci after treatment with G418 30 h post-transfection. Subconfluent 9-cm dishes of WAXI cells were transfected with plasmid DNA at $10 \mu\text{g}$ per dish and 30 h later were transferred to medium containing $400 \mu\text{g ml}^{-1}$ G418. Foci of rapidly proliferating cells were counted after 14 days. In dishes treated with the pSV-2-neo clone only, no foci were seen, and only occasional isolated senescent cells. Expt c, Anchorage and serum dependence of WAXI cells after transfection with p53 vectors and/or T24 Ha-ras1. The cell lines were assayed for anchorage-independent growth by suspension in 1.2% methylcellulose (Dow A4M) in DMEM plus 10% FCS cultured in 60-mm dishes over a base layer comprising 0.6% agarose in complete medium. The number of colonies present at 14 days was counted and expressed as a percentage of the total number of cells plated. ND, not determined.

* Occasional dishes show abortive colonies of senescent cells (see Fig. 1).

WAXI cells transfected with p4JJKan showed no evidence of crisis. Clones resistant to G418 were carried through 12 passages at a 1:10 split ratio and were examined for cloning efficiency in monolayer cultures. These cells had a high plating efficiency (65–70%) and furthermore, all individual colonies were morphologically identical. There was no evidence for the generation of senescent or terminally differentiated cells at any time after the initial outgrowth following transfection. Cloned immortalized WAXI cells maintained in complete medium showed a reproducible doubling time of 18 h. Our vector p4JJKan has also been examined for immortalizing activity in Syrian hamster dermal fibroblasts (R. Newbold, personal communication) with negative results. Interestingly, Newbold is also unable to induce immortalization of these cells with *myc* clones.

WAXI cells and the lines immortalized by the p53 constructs were transfected with cloned activated c-Ha-ras using pT24-Ha-ras-1, which contains a 6.6-kilobase *Bam*HI fragment of c-Ha-ras from the bladder cancer cell line T24, and were scored for transformed foci at day 21. Characteristic *ras* foci were seen only in the p4JJKan immortalized WAXI cells (Fig. 3); mortal

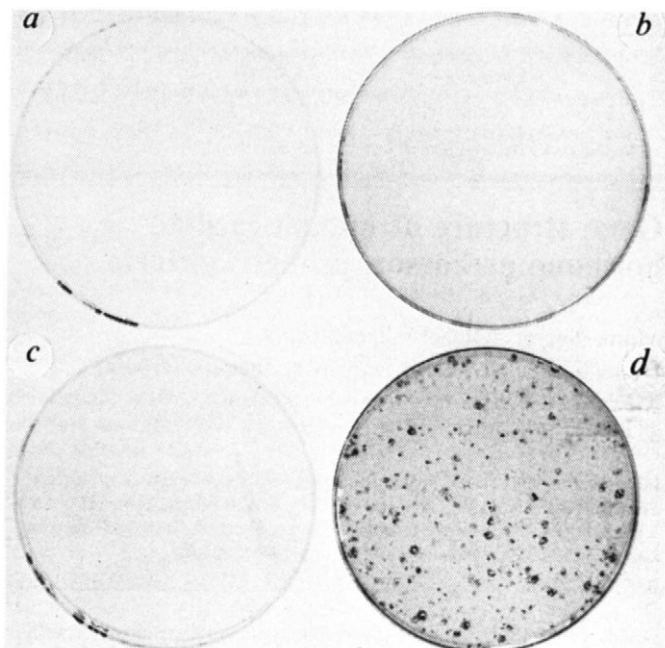


Fig. 3 Transforming activity of T24 Ha-ras1 on WAXI cells before and after immortalization by the p53 expression vector p4JJKan. *a*, WAXI cells; *b*, WAXI cells immortalized by p4JJKan; *c*, WAXI cells transfected with T24 Ha-ras1; *d*, p4JJKan-immortalized WAXI cells transfected with T24 Ha-ras1.

Methods: Monolayers of WAXI cells at passage 5, and p4JJKan-immortalized WAXI cells, established in Petri dishes at 2×10^5 cells per dish. The following day they were transfected with cloned T24 Ha-ras1, using the calcium phosphate method. $1 \mu\text{g}$ per 10^6 cells was used in each dish. After 21 days culture, characteristic *ras*-transformed foci were detected in the p4JJKan-immortalized WAXI cells only. Mortal WAXI cells showed no evidence of *ras* focus formation at any dose of T24 Ha-ras1 added (from 0.01 to $10 \mu\text{g}$ per dish).

WAXI cells were unaffected by the *ras* DNA. *ras*-transformed p53-immortalized WAXI cells were cloned and examined further. These cells show high anchorage-independent growth and rapid growth in low serum (Table 1). Furthermore, they induce tumours in nude rats. Immortal but untransformed WAXI cells are anchorage dependent and only grow in medium containing relatively high serum concentrations. Therefore, the major detectable phenotypic effect of the p53 expression construct p4JJKan is extension of cellular lifespan and it has no detectable effect on other phenotypes known to be associated with transformation. The observation that elevated levels of p53 are widely associated with malignant transformation induced by a variety of stimuli raises the possibility that this protein has a fundamental role in multi-stage carcinogenesis.

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Gene structure of human cardiac hormone precursor, pronatriodilatin

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Atrial cardiocytes contain granules typical of protein-secreting cells¹, and atrial extracts are known to contain a powerful natriuretic and diuretic activity² and to possess smooth muscle relaxant activity³. A variety of active atrial peptides have been isolated, including a family of related peptides showing natriuretic, diuretic and smooth muscle relaxant activities in rat⁴⁻⁹ and human atria¹⁰; these peptides were named atrial natriuretic factor (ANF). Another unrelated peptide from pig atria¹¹, cardiodilatin, is thought to possess only smooth muscle relaxant activity. Its partial amino acid sequence shows no homology with ANF sequences. The sequence analysis of a large form (106 amino acids) of ANF¹² and of ANF complementary DNA clones¹³⁻¹⁶ indicates that cardiodilatin and ANF peptides are synthesized from a common precursor. This precursor also contains a signal peptide sequence expected of a secretory protein¹⁷. We now describe the complete structure and sequence of the human gene for this novel hormone precursor that we call pronatriodilatin¹².

A rat pronatriodilatin cDNA clone¹³ was used as probe to isolate the human pronatriodilatin (hPND) gene from a human genomic DNA library¹⁸. A recombinant phage (λ hPND13) containing approximately 14 kilobases (kb) of human DNA was isolated. Restriction endonuclease cleavage sites were mapped in the human DNA by double digestions and fragments containing exon sequences were identified by hybridization with the rat pronatriodilatin cDNA probe. As the rat cDNA clone might be less homologous to human sequences in the untranslated regions, we localized exons containing these sequences with radioactive cDNA probes complementary to human poly(A)⁺ atrial RNA. A first probe made by priming cDNA synthesis with oligo-d(T)₁₂₋₁₈ was used to map the exon containing 3'-untranslated sequences. Hybridization of hPND gene restriction fragments with this probe also suggested the presence of a transcribed and possibly repeated sequence element in the large intron of the gene flanking the *Ava*I site. This was confirmed by hybridization of the same restriction fragments with nick-translated total human DNA. DNA sequence determination (see below) indicates that this region contains repeated sequences of the *Alu* family¹⁹. The first exon of the gene was localized using a second human cDNA probe made by priming with a synthetic eicosamer complementary to the previously determined sequence encoding amino acids 31-37 of the precursor.

The complete structural organization of the hPND gene is shown in Fig. 1. The 2.6-kb *Bam*HI-*Eco*RI fragment (Fig. 1b) contained all the hybridizing sequences and was subcloned in pBR327 for further characterization. DNA sequencing was performed by the chemical²⁰ and chain termination²¹ methods. For the latter, single-stranded templates were obtained by subcloning in M13mp8 and mp9 (ref. 22) (Fig. 1d). The hPND gene sequence, including about 500 base pairs (bp) upstream of the

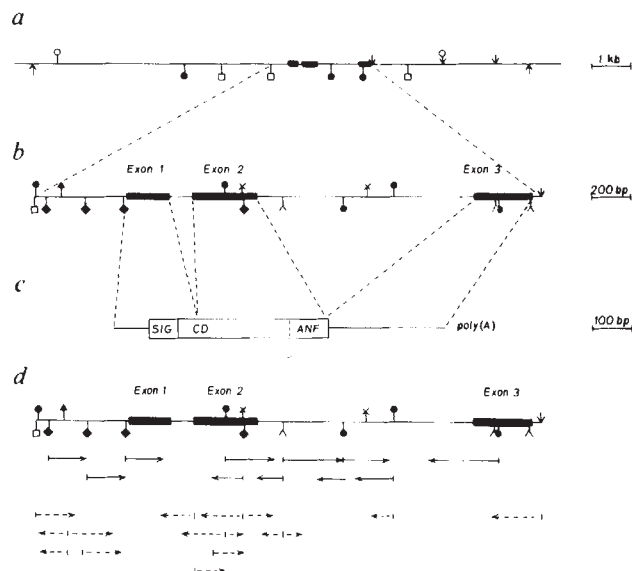


Fig. 1 *a*, Structure of human pronatriodilatin (hPND) gene. A map of restriction enzyme cleavage sites was obtained by double digestion of a bacteriophage λ Charon 4A recombinant containing ~14 kb of human DNA. Exon sequences (bars) were localized on this map by hybridization with various rat and human probes as outlined in the text. The following symbols are used for the restriction enzyme cleavage sites: *Eco*RI($\downarrow$), *Hind*III($\uparrow$), *Xba*I(∇), *Bam*HI($\square$), *Pst*I($\bullet$), *Ava*I($\circ$), *Kpn*I($\blacktriangle$), *Xho*I($\times$), *Pvu*II($\blacklozenge$) and *Nco*I(λ). *b*, Detailed restriction map of the 2.6-kb *Bam*HI-*Eco*RI fragment containing all the sequences hybridizing with pronatriodilatin cDNA probes. A subclone containing this fragment in pBR327 was used for sequencing. *c*, Schematic diagram of hPND mRNA showing regions present in each of the three exons. The translated portion of the mRNA is shown as an open box which includes the putative signal peptide sequence (SIG), the sequence homologous to pig cardiodilatin (CD) and the ANF sequence. The ANF sequence is preceded by a broken vertical line to indicate the putative nature of this processing site because many ANF peptides with variable-length amino termini have been described⁴⁻⁹. *d*, Strategy used for DNA sequence determination of hPND gene. Arrows below the diagram of the 2.6-kb *Bam*HI-*Eco*RI fragment indicate the length and direction of sequences obtained by the chain termination²¹ (solid arrows) or chemical²⁰ (broken arrows) methods. Single-stranded templates were obtained by subcloning in M13mp8 and mp9 (ref. 22) fragments of the 2.6 kb *Bam*HI-*Eco*RI produced with *Ava*I, *Ava*I + *Nco*I, *Pvu*II, *Pst*I or *Xho*II. Fragments were labelled at their 3' ends by filling-in²⁵ with the Klenow fragment of *Escherichia coli* DNA polymerase I for chemical sequence determination. The sites used for labelling were: *Ava*I, *Ava*II, *Bam*HI, *Eco*RI, *Fok*I, *Hin*FI, *Nco*I, *Sau*I.

Methods: A human genomic DNA library¹⁸ (kindly provided by Dr Tom Maniatis) was screened by filter hybridization²⁶ with a nick-translated²⁷ rat pronatriodilatin cDNA probe¹³. Hybridizations were performed at 55 °C in 20% formamide, 6 $\times$ SSC (20 $\times$ SSC is 3 M NaCl, 0.3 M sodium citrate), 0.05 M sodium phosphate buffer pH 6.8, 5 $\times$ Denhart's solution²⁸, 50 μ g ml⁻¹ sonicated denatured salmon sperm DNA. After hybridization overnight, the filters were washed at 50 °C in 1 $\times$ SSC and 0.1% SDS. Exon sequences were localized in the recombinant λ hPND13 by hybridization with the rat cDNA probe in the same conditions as above. The localization of 5' and 3'-untranslated sequences was ascertained with homologous human cDNA probes. These probes were synthesized in reactions containing 80-200 μ g ml⁻¹ human atrial poly(A)⁺ RNA, 400 μ g ml⁻¹ oligo-d(T)₁₂₋₁₈ or 6-7 μ g ml⁻¹ synthetic 20-mer (5'-AGGTCTGCGTTGGACACGGC-3') as primers, 200 U ml⁻¹ avian myeloblastosis virus reverse transcriptase and buffer (50 mM Tris-HCl pH 8.3, 60 mM KCl, 8 mM MgCl₂, 5 mM dithiothreitol).

transcription initiation site, is shown in Fig. 3 along with the translation product.

From a comparison of human¹⁶ and rat¹³⁻¹⁵ cDNA sequences, we conclude that the hPND gene is constituted of three exons. The first exon contains 5'-untranslated sequences, the initiator

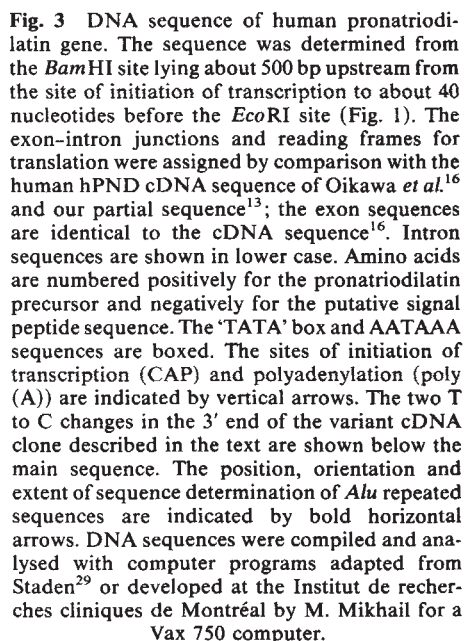
AUG codon, sequences for a 25-amino acid signal peptide and the first 16 amino acids of cardiodilatin; the second exon (327 bp) contains the remaining coding information up to the penultimate ANF amino acid; and the third exon (302 bp) contains the last codon for ANF, a stop codon and 3'-untranslated sequences. Altogether, the gene spans ~2 kb, including intron A (122 bp) and intron B (~1,050 bp).

The overall structural organization of the hPND gene is typical of a protein-coding eukaryotic gene. The intron sequences bordering the intron-exon junctions are homologous to the consensus²³ found at these junctions in other eukaryotic genes. Initiation of transcription often²⁴ occurs at an A residue about 30 nucleotides downstream from the 'TATA' box²³ sequence. The site of initiation of hPND gene transcription was determined by primer extension with the same synthetic oligomer used to locate the first exon. As shown in Fig. 2, the end of the major primer extension product corresponds to a G residue 24 bp downstream of the TATAAA sequence boxed in Fig. 3. Two minor bands would correspond to transcripts one nucleotide shorter and one nucleotide longer than the major species. Thus, the initiation of hPND transcription seems to be heterogeneous, the G residue being the major site of initiation. Another sequence²³ (GG₇CAAT) usually found 70-80 nucleotides upstream of the cap site is not readily identifiable in the hPND gene. The large intron seems to contain two complete 300-bp repeated sequences that are 87% homologous to a consensus *Alu* family sequence¹⁹. The repeated sequences are in a head-to-tail configuration.

A notable difference between human and rat pronatriodilatin cDNA sequences is produced by a single nucleotide change that converts an arginine codon into a stop codon. As described for a hPND cDNA¹⁶, our genomic sequence does not encode the



Fig. 2 Localization of hPND gene transcription initiation site. The oligonucleotide and method described in Fig. 1 legend were used to produce primer-extension products that extend to the 5' end of human atrial pronatriodilatin mRNA. The 20-mer was also used as primer for DNA sequencing on an M13 template containing the first exon of the hPND gene and upstream sequences. The sequencing ladder is used to determine the first nucleotide transcribed in the hPND mRNA. The sequence around the site of initiation appears below the sequencing gel. The sequence is labelled positively from the major sites of initiation of transcription.

[illegible]

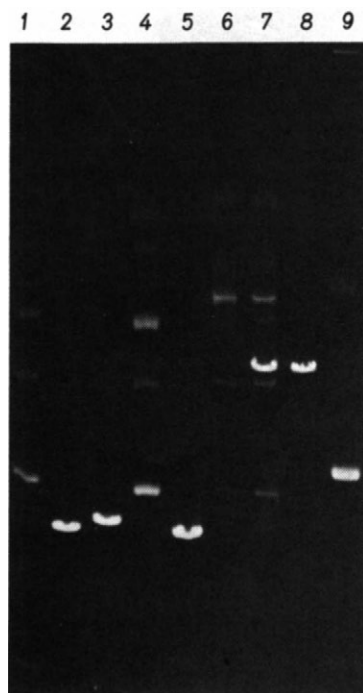


Fig. 4 Allelic variation in two hPND cDNA clones. The two hPND cDNAs were inserted in the *Pst*I site of pBR322 using dG:dC homopolymer tailing. Both cDNAs are about 350 bp, start within ANF-coding sequences and extend to the 3' end of hPND mRNA. In both cases, 200 bp were sequenced from the 5'-end to the *Pst*I site (underlined in Fig. 3) in the 3' non-coding region; only two nucleotide differences were found between the two cDNA sequences. One cDNA is identical to the genomic sequence (Fig. 3) and to the published hPND cDNA sequence¹⁶. One nucleotide difference in the stop codon of the variant cDNA makes it similar to the rat cDNA sequence¹³ and introduces a new *Kpn*I site in the cDNA as confirmed by the restriction enzymes digestions shown here. The two cDNA clones were characterized by electrophoresis on 0.8% agarose gel: lanes 1-3, variant cDNA; lanes 4-7, wild-type cDNA. Lanes 1, 4, supercoiled plasmids; lanes 2, 5, digestion with *Nco*I which cuts both cDNA inserts twice; lanes 3, 6, digestion with *Kpn*I; lane 8, same as lane 7 but including another plasmid linearized by *Kpn*I as internal control. This control plasmid is shown linearized by *Kpn*I in lane 8 and in supercoiled form in lane 9.

two arginine residues after the ANF sequence. In addition to hPND cDNA clones that are identical to the cloned genomic sequence, we have isolated a short cDNA clone (~350 bp) that, like the rat cDNA clones, encodes two arginine residues after the ANF sequence¹³. This cDNA clone differs at only two positions (within the 200 bp sequenced) from the other human sequences. The first nucleotide difference changes the stop codon into an arginine triplet and creates a new *Kpn*I restriction site in the cDNA clone (Fig. 4). The other nucleotide change is in the 3'-untranslated region; the two nucleotides that differ from the genomic sequence are shown on the complete sequence (Fig. 3). As Southern blot experiments (ref. 16 and our work, not shown) suggest the presence of only one hPND gene, these two clones are likely to represent different alleles of the hPND gene.

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Nucleotide sequence of the gene encoding human atrial natriuretic factor precursor

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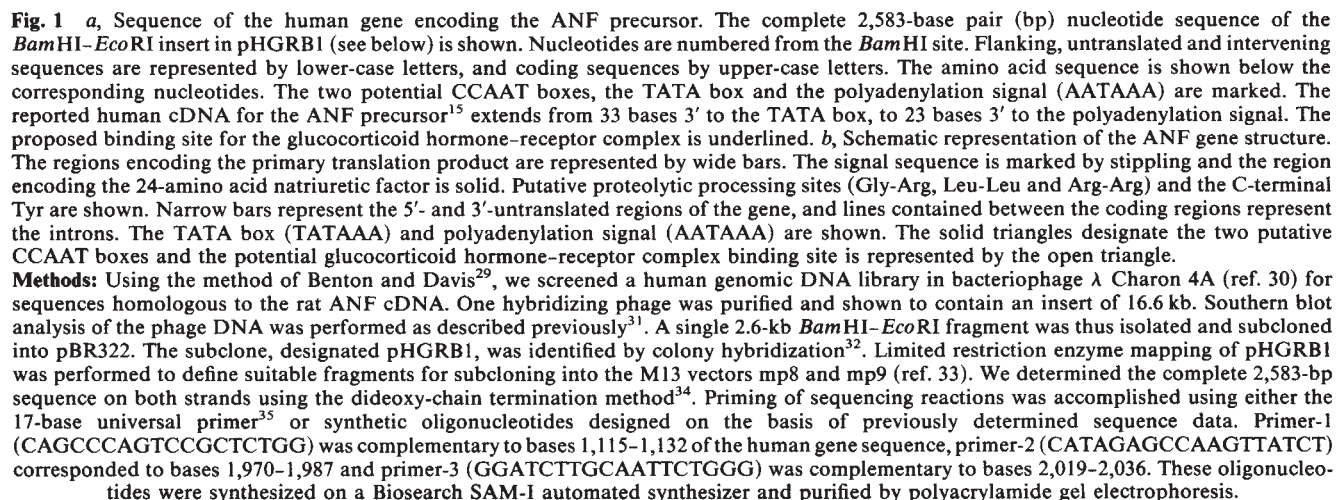
The mammalian atrium is an endocrine organ that may be involved in the control of blood pressure and extracellular fluid volume. A series of peptides¹⁻⁶, which seem to be associated with atrium-specific secretory granules, have potent natriuretic, diuretic and smooth muscle relaxant activities. Sequence determination of several of these peptides, which range from 21 to 126 amino acids long, shows that they form a family, derived from a common precursor⁶⁻¹². Rat and human complementary DNAs that encode the precursor to the various peptides, collectively called atrial natriuretic factors (ANFs), have been cloned¹³⁻¹⁷. Nucleotide sequencing showed that the ANFs are located at the C-terminus of a polypeptide of relative molecular mass 13,000. We describe here the isolation and characterization of the corresponding human gene. Two introns interrupt the gene; one is located in the region coding for the N-terminus of the precursor and the other separates the codon for the C-terminal tyrosine from the rest of the peptide.

Using a rat ANF cDNA¹³ as probe, we isolated a phage containing a 16.6-kilobase (kb) human genomic fragment. We have subcloned its complete hybridizing region, present on a 2.6-kb *Eco*RI-*Bam*HI fragment, and determined its nucleotide sequence (Fig. 1). The first exon contains the 5'-untranslated region, the signal peptide and the 16 amino acids following the signal peptidase cleavage site. The second exon encodes the rest of the propeptide except for the C-terminal tyrosine, which is found at the 5' end of the third exon. The sequences at each of the exon-intron junctions match the consensus splice site sequence¹⁸. A TATA box occurs at position 442 and two potential CCAAT boxes occur at positions 235 and 342 (ref. 19).

The second intron contains the sequence GCCATCTATT-GTCCT (positions 1,238-1,297), a potential binding site for the glucocorticoid hormone-receptor complex. Karin *et al.*²⁰ described the consensus binding site sequence $\text{CTGGT}^{\text{A}}\text{CA}^{\text{AA}}\text{TGT}^{\text{C}}\text{CT}$. Although the first four bases of the consensus do not match the putative binding site in the human gene, 10 of the last 11 do. This includes the hexanucleotide

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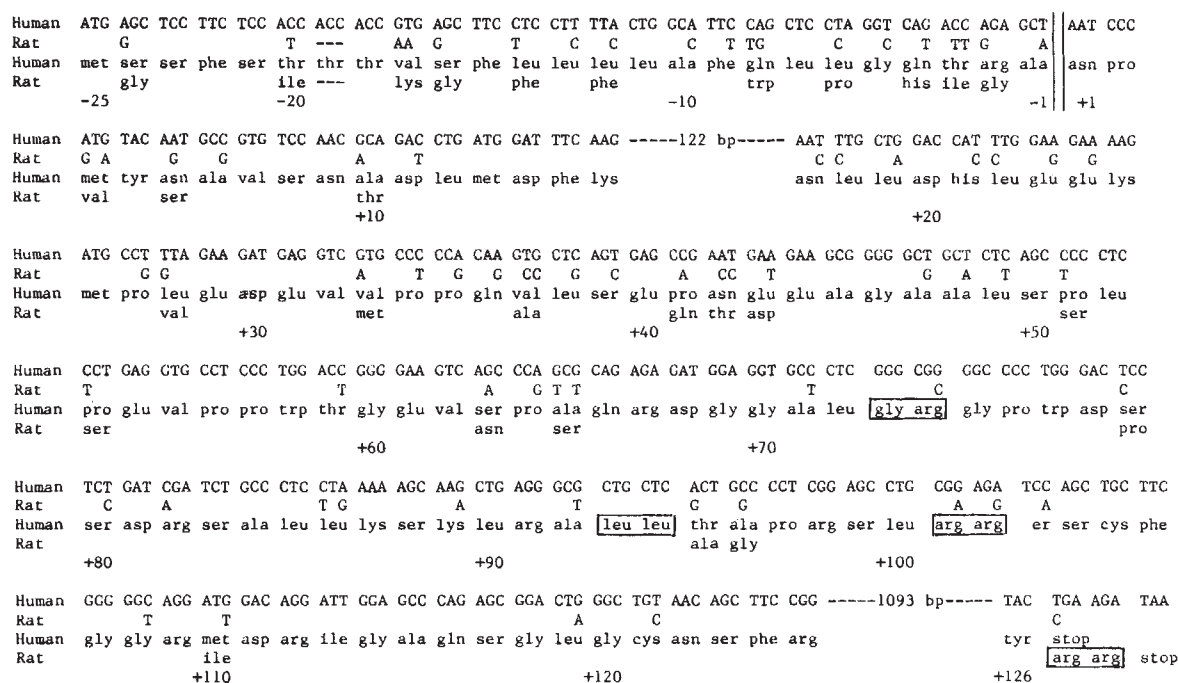


Fig. 2 Comparison of the human and rat sequences. The amino acid sequences of the human and rat ANF precursor are aligned with their corresponding nucleotide sequences. The rat sequences are shown only where they differ from the human sequences. Numbering refers to the human amino acid sequence, with +1 referring to the N-terminal Asn of the propeptide. Alignment of the rat and human sequences is perfect except at codon -19, which is not present in the rat precursor. Potential proteolytic processing sites^{10,12-17} are boxed. Rat sequences are from refs 13, 14 and 16.

sequence, TGT⁵CT, found in all glucocorticoid receptor binding sites^{20,21}. As with the human growth hormone gene, this site occurs within an intron²². Also within the second intron are two tandem *Alu* repeats, which are 76% homologous, spanning bases 1,356-1,646 and 1,647-1,942. Each shows a marked homology (70-86%) with the various *Alu* repeat sequences described by Deininger *et al.*²³.

Differences between the rat and human propeptide amino acid sequences (+1 to +126, Fig. 2) occur at 17 positions, giving an 86.5% amino acid sequence homology. The nucleotide sequence homology over the same region is 85.4%. The homology is much lower between the signal peptide sequences of the two species, as has also been observed in inter-specific comparisons of somatostatin and proopiomelanocortin genes²⁴⁻²⁶. In each case, however, the hydrophobic character of the signal peptide is preserved. Also, the human gene contains a single polyadenylation signal, AATAAA (ref. 27), whereas the rat cDNA has two^{13,14,16}.

Active peptide hormones are not usually interrupted by introns, in agreement with the suggestion that functional domains are defined by exons²⁸. The intron that occurs within the ANF coding sequence, however, separates the C-terminal tyrosine from the rest of the sequence. Note, in this context, that *in vitro* assays comparing the natriuretic and vasoactive properties of synthetic des-Tyr-ANF with native ANF have shown that the C-terminal tyrosine may not be required for biological activity¹. Comparison of the rat cDNA with the human gene structure suggests that the ANF gene may have evolved from a longer, possibly multi-functional gene. The C-terminal Arg-Arg in the rat may be a remnant of the original structure, and the single amino acid encoded in the third human exon may represent a further evolutionary step towards a two-exon gene.

The physiological role of the atrial natriuretic peptides is unclear. Several schemes by which the ANF precursor is processed to give short biologically active peptides have been proposed^{10,12-17}, but whether any of these occurs *in vivo* remains to be determined. Evidence has been recently obtained that ANF peptides circulate in dog plasma and that their levels correspond to variations in blood pressure (J.A.L., unpublished

results). Knowledge of the gene sequence for the human ANF precursor should now allow us to investigate the biological activities of various encoded peptides and to study their role in controlling human blood pressure and fluid volume.

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Identification of the coding sequence for a reverse transcriptase-like enzyme in a transposable genetic element in *Drosophila melanogaster*

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The largest group of transposable elements in *Drosophila melanogaster*, *copia*-like elements^{1,2}, share some important structural features with and are intimately related in evolution to vertebrate retroviruses²⁻¹². To further clarify the relationship between retroviruses and *copia*-like transposable elements, we set out to determine the complete nucleotide sequence of the genome of 17.6, which has long terminal repeats homologous in nucleotide sequence to those of avian leukaemia-sarcoma virus¹². We report here that 17.6 contains three long open reading frames comparable with *gag*, *pol* and *env* genes in retrovirus. At the level of amino acid sequence, the longest open reading frame of 17.6 includes a coding sequence similar to that for reverse transcriptase, suggesting a role for this enzyme in the life cycle of some *Drosophila copia*-like elements, analogous to the situation in retrovirus.

The nucleotide sequence of the internal body (the region flanked by long terminal repeats (LTRs)) of 17.6, inserted in histone genes in λ hist 17.6^{12,13}, was determined by the Maxam and Gilbert procedure¹⁴. The resultant nucleotide sequence was combined with the LTR sequences previously published¹² to give the nucleotide sequence of the 17.6 genome (7,439 base pairs (bp)): translation of the nucleotide sequence showed 17.6 to contain three long open reading frames (ORFs), 1, 2 and 3 (Fig. 1). Other ORFs (locations not shown here) are too short to encode the polypeptides of more than 150 amino acids. The majority of 17.6 elements distributed on the chromosome may contain the nucleotide sequences for these three long ORFs, as the restriction maps of more than half of the 17.6s isolated from 18 chromosomal loci were identical to the 17.6 map examined here¹⁵. In three 17.6s examined, including the one discussed here, no differences were found in the nucleotide sequence of a 300 bp region arbitrarily chosen within the internal body.

The ORFs 1, 2 and 3 of 17.6 show similarities in size and location to *gag*, *pol* and *env* genes, respectively, of avian leukaemia virus (ALV)¹⁷ and Moloney murine leukaemia virus (MoMuLV)¹⁶ (Fig. 3). Furthermore, as with the retrovirus proviruses, all these long ORFs of 17.6 were found to reside on a specific DNA strand simultaneously containing the nucleotide sequences corresponding to a Hogness box, polyadenylation signal, primer binding site and polypurine sequence in ALV¹² (see Fig. 1). A 540-bp noncoding sequence, very rich in A+T content, was also located upstream from the ORF 1 (Fig. 1). As shown below, the ORF 2 of 17.6 includes the coding sequence for the enzyme similar to reverse transcriptase, which, in the case of the retrovirus, is coded by *pol*.

Virus-like particles (VLPs) with *copia* RNA and the reverse transcriptase-like enzyme activity have been identified in *D. melanogaster* and *copia* RNA encapsidated is considered to have a weak mRNA activity for making the polypeptides immunologically related to the VLP coat proteins¹¹. However, it is not clear whether the *copia* genome contains the gene for the reverse transcriptase-like enzyme. This fact, along with the above finding that 17.6 is similar in genome organization to retroviruses, prompted us to survey the coding sequence for the

enzyme similar to reverse transcriptase of retrovirus within the 17.6 genome. We compared the amino acid sequences of the putative polypeptides expected to derive from the three ORFs of 17.6 with those of the reverse transcriptases of MoMuLV¹⁶ and Rous sarcoma virus (RSV)¹⁷, and that of the putative reverse transcriptase of cauliflower mosaic virus (CaMV)¹⁹. The ORF 2 protein of 17.6 was found to contain a 200–300 amino acid long domain which could match some portions of the *pol* gene products of MoMuLV and RSV, and of the counterpart of CaMV (Fig. 2). The amino acid sequence homology between 17.6 and MoMuLV was estimated to be 25%, whereas those between 17.6 and RSV and between 17.6 and CaMV were 20 and 30%, respectively. We will hereafter refer to the regions with these homologous amino acid sequences and their DNA counterparts as RT polypeptides and RT DNAs, respectively.

It is noteworthy that the RT polypeptides of MoMLV and RSV correspond to the regions containing the amino acid sequences which have been suggested as being conservative among various reverse transcriptases and similar enzymes, and in charge of the polymerase activity²⁷ (Fig. 3). Furthermore, the RT polypeptide of 17.6 was found to contain 9 of the 10 amino acids that are invariant in all reverse transcriptases so far examined²⁷ (Fig. 2). In addition, the relative positions of the RT DNAs of 17.6, MoMLV and ALV (RSV), were found to be similar to one another, assuming the ORF 2 of 17.6 to be identical to the *pol* genes of the retroviruses (Fig. 3). Thus, it is probable that the RT DNA of 17.6 is the major coding sequence for the enzyme similar to the reverse transcriptase of retrovirus. In a separate experiment (unpublished), we determined the nucleotide sequences of the entire 297 (ref. 6) genome, and a portion of the 412 (ref. 3) genome and found that these elements also contain the nucleotide sequences corresponding to the RT DNA. Thus the nucleotide sequence of the RT DNA seems to be conserved among at least some classes of *Drosophila copia*-like element; the RT polypeptides have played an important role in maintaining these elements as transposable elements during evolution.

The carboxyl-terminal regions of the reverse transcriptases of ALV and MoMuLV contain DNase activity^{16,18,20}, probably required for integration²⁸. Interestingly, the ORF 2 proteins of 17.6 and 297 contain the regions with highly conservative amino acid sequences near their carboxyl ends (unpublished data). We have identified recently the significant amino acid sequence homology between these carboxyl terminal domains of ORF 2 proteins of 17.6 and 297, and the DNase regions of ALV and MoMLV, suggesting that even the overall organization of these genes is similar to that of the *pol* genes of retroviruses (manuscript in preparation). ORF 1 of 17.6 and 297 may be related also to *gag* of MoMLV, as weak homology at the level of amino acid sequence could be detected.

17.6 contains nucleotide sequences that apparently correspond to a primer binding site and polypurine sequence, both mandatory for the initiation of DNA synthesis in reverse transcription in a retrovirus system¹². As described above, we have confirmed that 17.6 has the coding sequence for reverse transcriptase-like enzyme. Furthermore, *Drosophila* appears to contain a candidate tRNA of 297 primer since our data (unpublished) have revealed that as with retrovirus primer tRNA^{16,17}, the 3'-terminal 18-bp fragment of one of the *Drosophila* serine tRNAs is complementary to the 18-bp, putative primer binding site of 297. Circular DNAs of 17.6 and 297 which appear to correspond to the circular retrovirus DNAs²¹ have also been identified in *Drosophila* (ref. 10 and our unpublished data). Thus, it is quite conceivable that the RNAs of 17.6 and other similar elements are reverse transcribable by a mechanism similar to that of retrovirus, and that the resultant DNAs or their derivatives are used as intermediates for integration. VLPs, which may be required for the effective reverse transcription, have been found only in the case of *copia*.

Our results support the view that *copia*-like transposable elements in *Drosophila* and retroviruses in vertebrates bear a very close evolutionary relationship, but do not reveal whether

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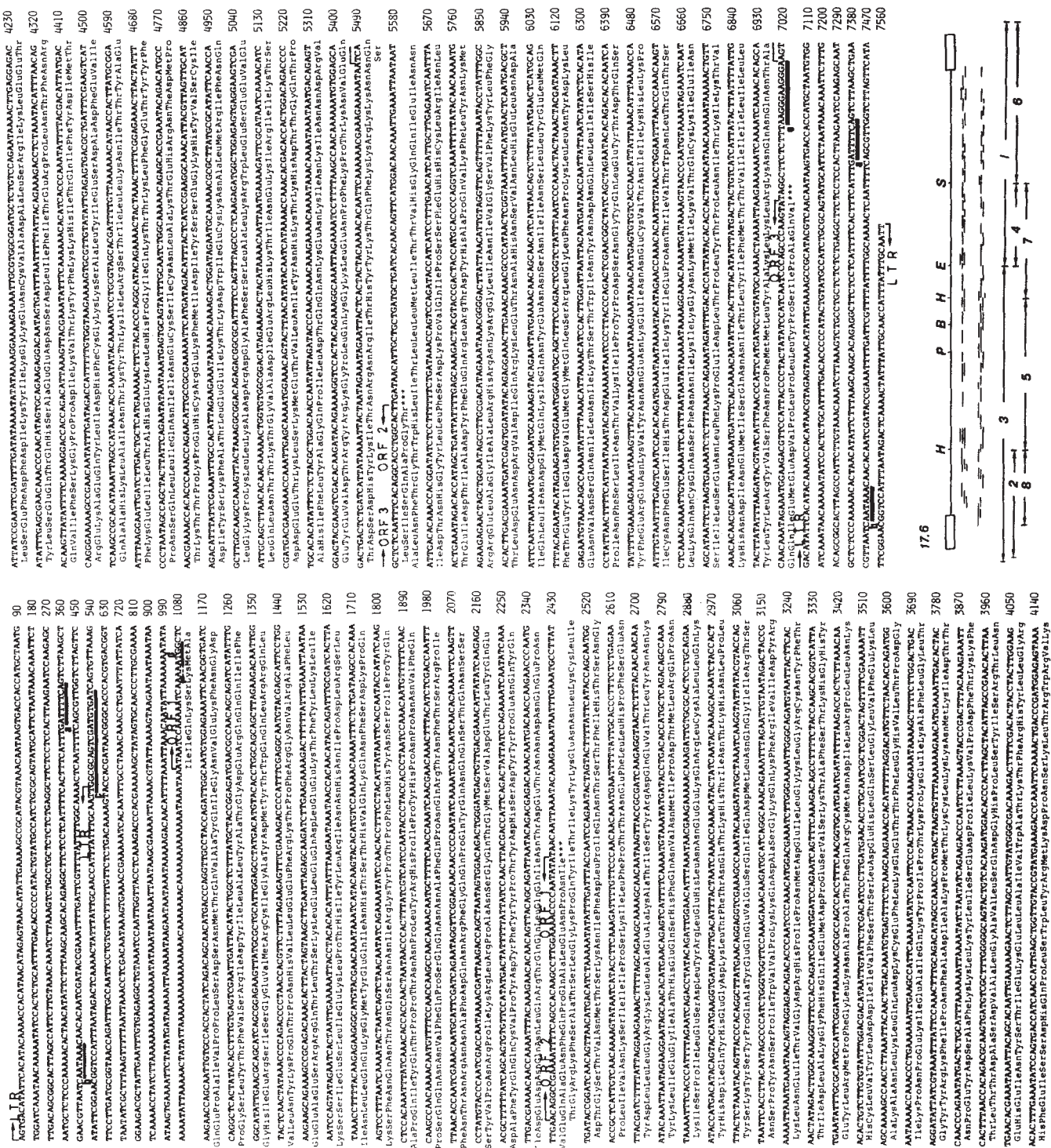


Fig. 1 The complete nucleotide sequence of the 17.6 genome, presented as the putative sense strand which includes three open reading frames. The ranges of three ORFs, which are translated below the nucleotide sequence, are shown by L-shaped arrowheads. *, The termination codon of each ORF; underlines, nucleotide sequences corresponding to a, the Hogness box, b, polyadenylation signal, c, primer binding site and e, polypurine sequence in ALV¹². The underline labelled d shows the initiation signal for translation²³ of ORF 1.

Methods: The DNA extracted from λ hist 17.6 (containing a histone gene repeat inserted by 17.6 element) was digested by various restriction enzymes and the resultant DNA segments containing 17.6 pieces were recloned using pUC⁹⁵ as a cloning vector. The sizes and locations of the cloned DNA segments are shown by thick lines with arrowheads and numbers in the scheme on the right. After *Escherichia coli* cells [(JM83 ref. 24) or GM48 (dcm-6, dam-3) (ref. 25) harbouring a desired plasmid] were cultured in 100 ml L broth for 15h at 37 °C with shaking, plasmid DNA was extracted according to Clewell and Helinski²⁶ and purified by banding in a CsCl gradient containing 50 μ g ml⁻¹ of ethidium bromide. Closed circular DNA was collected, treated with 5 M NaCl-saturated isopropanol several times, dialysed against 1 mM Tris-HCl (pH 7.4) + 0.1 mM EDTA and stored at 4 °C until use. Plasmid DNA was digested with a suitable restriction enzyme, end-labelled with either kination or filling-in, redigested with the second restriction enzyme and sized on agarose or polyacrylamide gels by electrophoresis. The desired DNA segment extracted was then subjected to the partial chemical degradation method of Maxam and Gilbert¹⁴. The overall strategy for sequencing is depicted by thin underlines in the lower margin illustration. About 90% of the final sequence was determined for both strands of duplex DNA to minimize artefacts such as compression; upper and lower lines show the regions of the DNA strands sequenced. B, *EcoRI*; H, *HindIII*; P, *PstI*; S, *SalI*.

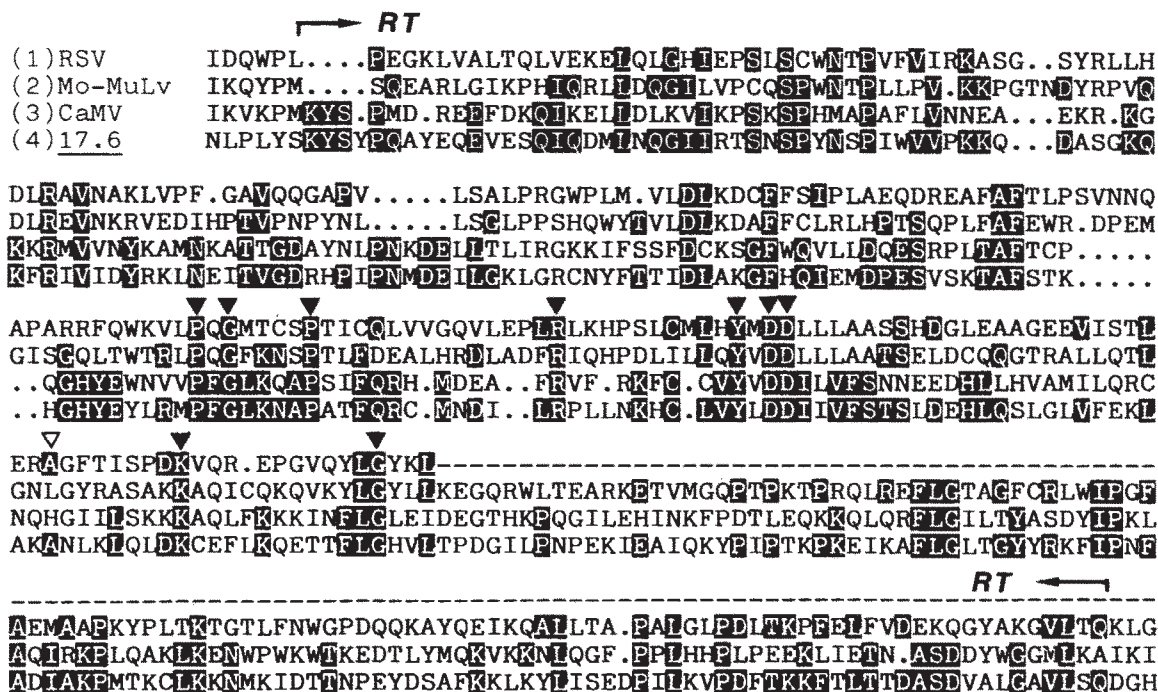


Fig. 2 Comparison of the amino acid sequences of RT polypeptides from various sources. The amino acid sequences were deduced from the nucleotide sequences (RSV, ref. 17; MoMLV, ref. 16; CaMV, refs 19, 27; 17.6, Fig. 1 (this work)). L-shaped arrowheads labelled RT, ranges of the RT polypeptides. Lines 1, 2, 3, 4; amino acid sequences of the RT polypeptides of RSV, MoMLV, CaMV and 17.6, respectively. White letters enclosed by filled boxes, homology to 17.6 in lines 1, 2 and 3, and homology to RSV, MoMLV or CaMV in line 4. Filled triangles, locations of 9 of the 10 invariant amino acids²⁷ found in 17.6; an open triangle, amino acid not found as an invariant in 17.6.

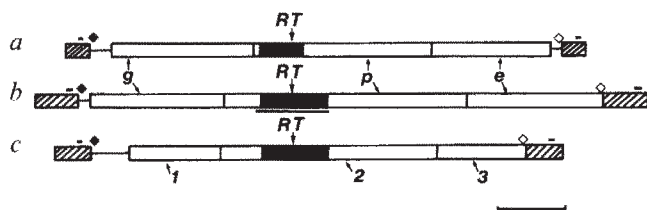


Fig. 3 Comparison of the genome structures between retrovirus proviruses and a copia-like element, 17.6. *a*, *b*, Genome organizations of the integrated proviral forms of ALV and MoMLV, deduced from the RNA genome structures^{16,17}, respectively. In particular, the structure of the ALV provirus is identical to that of RSV without *src* gene and one DR¹⁷. The structure of 17.6 (2) was deduced from the nucleotide sequence shown in Fig. 1. Hatched boxes, LTRs; filled boxes, RT DNAs (see Fig. 2); open boxes, ORFs of 17.6 and the genes of retroviruses. Filled lozenges, positions of the primer binding sites in *a* and *b* and the putative primer binding site in *c*; open lozenges, positions of polypurine sequences; small rectangles over the hatched LTR boxes, regions containing the Hogness box and polyadenylation signal or their related sequences^{12,16,17}. Scale bar, 1,000 bp. Arrows labelled RT, positions of the nucleotides corresponding to the most conservative amino acid sequences, YXDD, in the RT polypeptides (see Fig. 2). Underline in *b*, region of amino acid sequence highly homologous to that of the reverse transcriptase-like enzyme of CaMV (see ref. 27). *g*, *gag*; *p*, *pol*; *e*, *env*; 1, ORF 1; 2, ORF 2; 3, ORF 3.

the common ancestor is a retrovirus^{11,12} or a transposable element²².

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Nicotinic receptor stimulation activates enkephalin release and biosynthesis in adrenal chromaffin cells

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Neuroendocrine cells release a portion of their stored secretory hormone content when exposed to tissue-specific secretagogues. In the case of the adrenal medulla, catecholamines and enkephalin peptides, as well as other secretory proteins, are secreted in response to acetylcholine, which is released onto cholinergic recep-

tors on chromaffin cells upon splanchnic nerve stimulation *in vivo*¹⁻³. Secretagogue stimulation thus depletes intracellular stores of exportable hormone. We were interested to know whether the signal for exportable hormone release might also function as a signal for compensatory hormone repletion by enhancing the biosynthesis of the released hormone(s). Accordingly, we have investigated the effect of nicotinic receptor stimulation on Met-enkephalin peptide biosynthesis and expression of proenkephalin messenger RNA in primary cultures of bovine chromaffin cells. Our results, reported here, suggest a model for stimulus-secretion-synthesis coupling in which nicotinic receptor occupancy activates two pathways. One pathway, dependent on calcium and not mimicked by increased intracellular cyclic AMP, leads to exocytotic hormone release; the other, probably via a calcium-dependent increase in intracellular cyclic AMP, leads to a compensatory increase in intracellular enkephalin through activation of transcription of the proenkephalin structural gene.

Chromaffin cells were dissociated from bovine adrenal medulla and maintained in primary culture for 72–96 h⁴. Medium was then removed from the cells and replaced with fresh medium containing nicotinic agonists and/or antagonists. Exposure to 10 μ M nicotine for 24–72 h resulted in a gradual increase in total Met-enkephalin immunoreactivity in the cultures (Fig. 1). This increase was completely blocked in the presence of 5 μ g ml⁻¹ cycloheximide (data not shown). It was preceded by an increase in mRNA coding for proenkephalin (mRNA^{enk}) which is already maximal at 24 h, when peptide levels begin to rise (Fig. 1). Nicotinic induction of enkephalin biosynthesis was maximal at the dose used (5–10 μ M) and was not observed with doses of nicotine $\geq$ 1,000 μ M. This desensitization to nicotine is also characteristic of nicotine-induced enkephalin and catecholamine release^{2,4}. The induction of mRNA^{enk} by nicotine is quite rapid: an increase in mRNA^{enk} is detectable within 2 h of exposure to 5 μ M nicotine and is maximal by 8 h (Fig. 2). This rapid time course suggests that nicotine is acting to increase mRNA^{enk} by enhancing proenkephalin gene transcription rather than by inhibiting its degradation. Enhanced transcription of the proenkephalin gene is also suggested by a rapid increase in nuclear proenkephalin precursor RNA following exposure to nicotine (H.-U.A., unpublished observations). Nicotine first causes an actual depletion of intracellular Met-enkephalin, due to its release into the culture medium. By 25 h, however, intracellular peptide stores have returned to pre-stimulation levels, indicating that compensatory biosynthesis has occurred (Fig. 2).

Nicotinic stimulation of Met-enkephalin biosynthesis, enkephalin release and levels of mRNA^{enk} are all blocked by addition of hexamethonium, a nicotinic antagonist (Table 1). In addition, induction of mRNA^{enk} by 10 μ M nicotine is blocked by hexamethonium added as long as 1 h after nicotine, suggesting that in these experimental conditions nicotinic induction of enkephalin biosynthesis is not secondary to a feedback effect of secretory products (for example, enkephalin, ATP, catecholamines); these are maximally released from the cells during the first 15 min of exposure to nicotine (L.E.E., unpub-

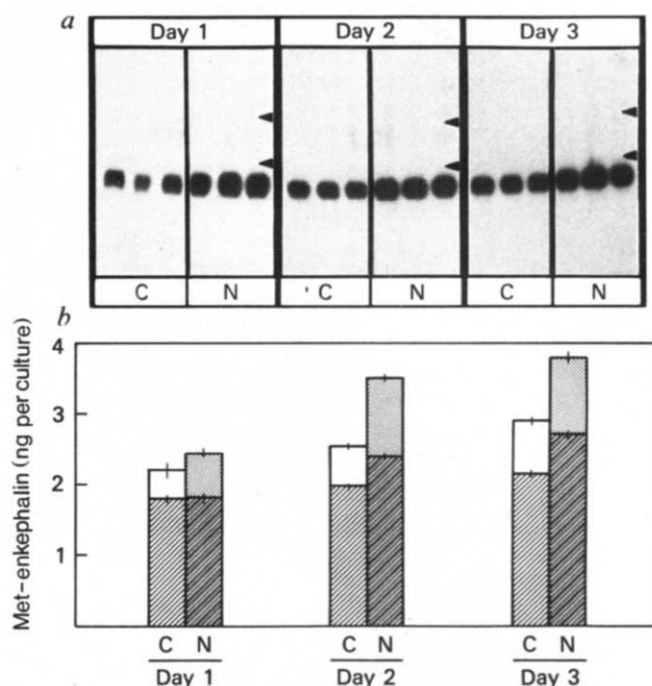


Fig. 1 Effect of chronic exposure to nicotine on mRNA^{enk} and immunoreactive Met-enkephalin in cultured chromaffin cells. **a**, Autoradiograms of Northern blots prepared from chromaffin cell total nucleic acids 1, 2 or 3 days following exposure to 5 μ M nicotine (N) or vehicle (C) and hybridized with a ³²P-labelled 400-base-pair *Pst*I restriction fragment of bovine proenkephalin A cDNA¹³ as described previously^{5,6}. Arrows indicate positions of 28S and 18S RNA. Autoradiograms were scanned densitometrically and corrected for total RNA applied in each sample by scanning densitometrically negative photographs of UV-transilluminated, ethidium-stained gels run in parallel to the gels used for Northern transfer. These values were (control at each time point corrected to 100%): day 1, C = 100 $\pm$ 5, N = 152 $\pm$ 10; day 2, C = 100 $\pm$ 6, N = 145 $\pm$ 8; day 3, C = 100 $\pm$ 9, N = 183 $\pm$ 22, $\bar{x} \pm$ s.e.m., $n = 3$. **b**, Immunoreactive Met-enkephalin was measured by radioimmunoassay using the RB-4 antiserum exactly as described in ref. 6. N, values measured in nicotine-treated cultures; C, values measured in untreated cultures. The hatched portion of each bar represents immunoreactive Met-enkephalin contained in the cells and the unhatched portion the immunoreactive-Met-enkephalin released into the medium. The total height of each bar represents total (cell + medium) immunoreactive Met-enkephalin. Values are the mean $\pm$ s.e.m. of three determinations (wells). Lower s.e.m. is that associated with cell content and upper s.e.m. is that associated with total (cell + medium) immunoreactive Met-enkephalin. **Methods.** Cells were prepared and maintained in culture at a density of 300,000 cells per 16-mm well as described previously⁴. On day 3 of culture, medium was removed and replaced with fresh medium (C) or medium containing 5 μ M nicotine (N). 24, 48 and 72 h later, parallel cultures were extracted for total nucleic acids or enkephalin peptides as described elsewhere⁵.

Table 1 Effect of hexamethonium blockade on nicotine-stimulated Met-enkephalin metabolism and increased cellular cyclic AMP in cultured chromaffin cells

1	2	3	4	5
Additions	mRNA ^{enk} % of control	Met-enkephalin (ng per well)	Met-enkephalin (% release)	Cyclic AMP (pmol per well)
None	100 $\pm$ 3.6	2.54 $\pm$ 0.211	1.55 $\pm$ 0.398	1.19 $\pm$ 0.025
Nicotine	214 $\pm$ 18.5*	3.40 $\pm$ 0.147*	22.0 $\pm$ 0.41*	1.73 $\pm$ 0.066*
Hexamethonium	128 $\pm$ 5.6	2.53 $\pm$ 0.190	1.63 $\pm$ 0.071	1.32 $\pm$ 0.178
Nicotine + hexamethonium	138 $\pm$ 5.1†	2.33 $\pm$ 0.287†	5.18 $\pm$ 0.398*†	1.43 $\pm$ 0.050*†

Parameters measured 24 h (column 2) or 48 h (column 3) after exposure to 10 μ M nicotine and/or 50 μ M hexamethonium (added to the cultures 15 s before addition of nicotine) or 1 h (columns 4, 5) after addition of 10 μ M nicotine with simultaneous addition of 10 μ M hexamethonium (when present). Cyclic AMP was measured with a commercially available kit (NEN) by direct radioimmunoassay. Values represent the mean $\pm$ s.e.m. of 3 (cols 2, 5), 4 (col. 4) or 6 (col. 5) determinations (wells).

* $P < 0.01$ compared with control (no additions); † $P < 0.05$ compared with nicotine alone, using Student's two-tailed *t*-test.

lished observations). Muscarine has no effect on enkephalin release or biosynthesis at concentrations up to 200 μ M (data not shown).

The induction of enkephalin mRNA by nicotine is preceded by an increase in intracellular cyclic AMP which is also blocked by hexamethonium (Table 1). We have previously reported that forskolin and cholera toxin, which increase intracellular cyclic AMP, also increase mRNA^{enk} in chromaffin cells^{5,6}. Elevation of intracellular cyclic AMP has also been demonstrated to result in increased transcription of the prolactin gene in a clonal line of pituitary tumour cells⁷. Interestingly, elevation of intracellular cyclic AMP by forskolin neither stimulates acute release of Met-enkephalin (or catecholamines) from chromaffin cells nor potentiates the releasing effect of a submaximal dose of nicotine (L.E.E., unpublished data), consistent with a previous report⁸ of a lack of effect of 8-bromo-cyclic AMP or cholera toxin on catecholamine release from cultured chromaffin cells. In addition, forskolin-induced enkephalin mRNA elevation is not

dependent on calcium influx whereas the increase in mRNA^{enk} elicited by 5 μ M nicotine is markedly so, being totally abolished in the presence of the calcium channel blocker D-600 (control mRNA^{enk}, 100 $\pm$ 30.4%; 25 μ M forskolin, 237 $\pm$ 15.6%; 25 μ M forskolin plus 10 μ M D-600, 290 $\pm$ 25.3%; 5 μ M nicotine, 237 $\pm$ 15.6%; 5 μ M nicotine plus 10 μ M D-600, 110 $\pm$ 5.8%; data obtained from Northern hybridization and normalized as described in Fig. 1 legend). The calcium dependence of nicotinic induction of mRNA^{enk} may reflect a calcium requirement for activation of adenylate cyclase, as described for other systems in which secretagogues cause an elevation of intracellular cyclic AMP^{9,10}, or it may be necessary for the translocation of other messengers that ultimately affect enkephalin gene expression.

These data suggest a model for stimulus-secretion-synthesis coupling¹¹ in which secretagogue receptor activation causes both calcium-dependent secretion and a calcium-dependent increase in mRNA coding for the secreted peptide. The third messenger for stimulation of enkephalin biosynthesis may be cyclic AMP, acting via cyclic AMP-dependent protein kinase to enhance enkephalin gene transcription, as suggested for trans-synaptic induction of tyrosine hydroxylase in adrenal medulla^{8,12}.

It will be interesting to determine whether this mode of regulation also operates in other types of neural cells that store and secrete enkephalin as well as other neuropeptide hormones.

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The yeast ubiquitin gene: head-to-tail repeats encoding a polyubiquitin precursor protein

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Ubiquitin¹, a 76-residue protein, occurs in cells either free or covalently joined to a variety of protein species^{2,3}, from chromosomal histones⁴⁻⁶ to cytoplasmic proteins. Conjugation of ubiquitin to proteolytic substrates is essential for the selective degradation of intracellular proteins in higher eukaryotes^{7,8}. We show here that a protein homologous to human ubiquitin exists in the yeast *Saccharomyces cerevisiae*, and that yeast extracts conjugate human ubiquitin to a variety of endogenous proteins in an ATP-dependent reaction. We have isolated the *S. cerevisiae* ubiquitin gene and found it to contain six consecutive ubiquitin-coding repeats in a head-to-tail arrangement. This apparently unique gene organization suggests that yeast ubiquitin is generated by processing of a precursor protein in which several exact repeats of the ubiquitin

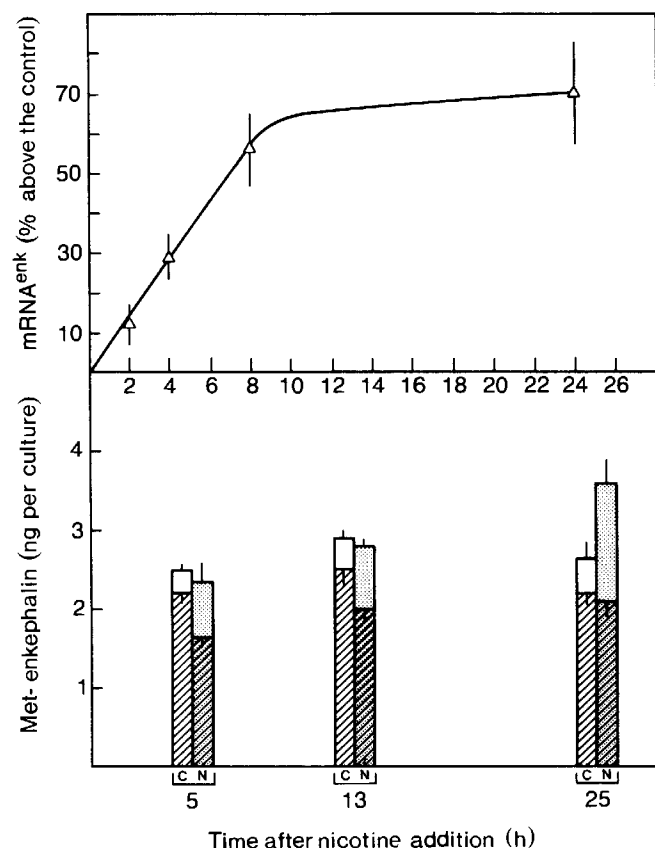


Fig. 2 a, Time course of mRNA^{enk} induction by nicotine; b, enkephalin immunoreactivity. The hatched and unhatched portions of the shaded and unshaded bars are as described in Fig. 1 legend.

Methods. Chromaffin cells were exposed to nicotine on day 3 of culture. At the times indicated, cultures were extracted for total nucleic acids or enkephalin peptides. a, Total nucleic acids were prepared as described previously^{5,6}, and the extract from 90,000 cells (~1 μ g of RNA) in 100 μ l of denaturing buffer was blotted onto Gene-Screen Plus paper (NEN) soaked in 20 $\times$ SSC in a Schleicher and Schuell Slot Blot apparatus. Blots were washed with 1 M sodium phosphate pH 6.5, air-dried and hybridized with the bovine enkephalin cDNA probe (see Fig. 1 legend). Blots were autoradiographed and each sample was then cut from the paper and radioactivity counted by liquid scintillation spectrometry in 10 ml of Hydrofluor (NEN). Values are expressed as per cent increase over the untreated control signal at each time point, and represent the mean $\pm$ s.e.m. of three individual determinations (wells). b, Enkephalin immunoreactivity was measured in culture medium and cell extracts at 5, 13 and 25 h after exposure to nicotine. Values are the mean $\pm$ s.e.m. of six determinations (wells).

amino acid sequence are joined directly via Gly-Met peptide bonds between the last and first residues of mature ubiquitin, respectively. Ubiquitin-coding yeast DNA repeats are restricted to a single genomic locus; although the sequenced repeats differ in up to 27 of 228 bases per repeat, they encode identical amino acid sequences. As this predicted amino acid sequence differs in only 3 of 76 residues from that of ubiquitin in higher eukaryotes, ubiquitin is apparently the most conserved of known proteins.

We identified recently the first mutant in the ubiquitin pathway⁹, and have used this mouse-derived cell cycle mutant, ts85, to directly demonstrate the ubiquitin dependence of selective protein degradation in higher eukaryotes⁷. A deeper analysis of the ubiquitin pathways by methods of molecular genetics should be possible if yeast possesses a functional ubiquitin system. Figure 1A shows that yeast contain a protein homologous to ubiquitin. This protein is specifically immunoprecipitated with an antibody to human ubiquitin, and is indistinguishable from ubiquitin when analysed electrophoretically on 18% polyacrylamide-SDS gels (Fig. 1A). Moreover, crude *S. cerevisiae* extracts catalyse the conjugation of added human ubiquitin to a variety of endogenous proteins in an ATP-dependent reaction (Fig. 1B, lanes d-h), as do mammalian cell-free systems (Fig. 1B, lane c) and intact cells^{7,9-12}.

The recently developed λ gt11 expression vector^{13,14} is suited for cloning genes whose products can be visualized by filter immunoassay. A yeast genomic DNA library carried in λ gt11 was screened using affinity-purified antibody against human ubiquitin, and a specifically immunoreactive phage, λ UB, was plaque-purified to homogeneity (see Fig. 2A legend for details). The nucleotide sequences at both ends of the λ UB insert were determined using the method of Maxam and Gilbert¹⁵. However, we found stop codons in all three reading frames and no potential ubiquitin-coding sequences. This suggests that vector-insert fusion proteins were not responsible for recognition by the anti-ubiquitin antibody.

To identify the immunoreactive coding sequences of λ UB, its 2.4 kb insert was sheared by sonication and subcloned into the pUC13 plasmid expression vector¹⁶ (Fig. 2A). An immunoreactive subclone designated pUB1 was purified, and the nucleotide sequence of its 240 base pair (bp) insert was determined. As shown in Fig. 2A, this DNA fragment does indeed encode an amino acid sequence identical to the C-terminus of human ubiquitin, from residue 44 to the terminal residue 76. Surprisingly, however, the predicted amino acid sequence continues without stop codons far past the presumptive ubiquitin C-terminus; these downstream sequences correspond in turn to the N-terminus of human ubiquitin (Fig. 2A).

The striking organization of the pUB1 insert (Fig. 2A) suggests that yeast ubiquitin is generated by processing of a precursor protein containing two or more precisely joined repeats of the canonical amino acid sequence of ubiquitin. Using the pUB1 insert as a probe, ubiquitin-coding sequences of the λ UB insert were mapped by Southern hybridization¹⁷ (data not shown), and found to span approximately 1.4 kb internal to the 2.4 kb λ UB insert, consistent with the possibility that λ UB contained a string of six 228-bp ubiquitin-coding repeats. The λ UB insert was subcloned into pBR322 (to yield the plasmid pUB2; see legend to Fig. 3), end-labelled as shown in Fig. 3A, and partially digested with either *Msp*I or *Mbo*II. This produced an electrophoretic 'ladder' of end-labelled products, as predicted by the model of multiple, precisely joined repeats of the 228-bp ubiquitin-coding elements (Fig. 3B and data not shown; see also Fig. 2A, B). Based on such restriction site periodicities, the ubiquitin gene appears to contain six tandem ubiquitin-coding repeats. An independent estimate of six repeats can be made on the basis of Southern hybridization analysis of either *S. cerevisiae* genomic DNA or the 2.4 kb pUB2 insert fully digested with *Dde*I and probed with pUB1 insert (see Fig. 3C). Figure 3C also shows that no major rearrangements of ubiquitin-coding sequences occurred during cloning of the pUB2 insert from yeast genomic DNA. A quantitative hybridization analysis, using DNA from λ UB as a titration standard, indicates that

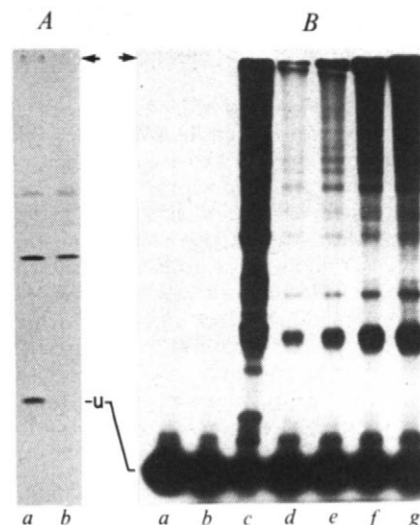


Fig. 1 Presence of a ubiquitin homologue in *S. cerevisiae*, and ATP-dependent conjugation of human ubiquitin to endogenous proteins in *S. cerevisiae* extracts. **A**, yeast cultures (strain S288C) in exponential growth were labelled with ^3H -lysine in glucose-supplemented SD medium for 30 min at 30 °C. Cells were pelleted by centrifugation, resuspended in 1 mM dithiothreitol (DTT), 10 mM Tris-HCl (pH 7.5), lysed using glass beads, and centrifuged at 100,000g for 1 h. The supernatant was heated at 80 °C for 10 min and clarified by centrifugation. Equal aliquots of the supernatant were immunoprecipitated (using fixed *S. aureus*³⁰) with either rabbit antiserum against human ubiquitin or an equal amount of preimmune rabbit serum (lanes a and b, respectively). Immunoprecipitates were analysed by electrophoresis in an 18% discontinuous polyacrylamide-SDS gel and fluorography⁹. Identical results were obtained in the absence of heat pretreatment, i.e., no additional protein species were specifically immunoprecipitated by the anti-ubiquitin antibody. u denotes the ubiquitin band. **B**, Strain S288C cells in exponential growth were lysed and centrifuged as above, and the supernatant used as a source of ubiquitin-conjugating activity. 120 μg of extract were assayed at 37 °C in a final volume of 30 μl by adding 3 μl (5×10^5 c.p.m.) of human ubiquitin (purified and labelled with ^{125}I as described previously⁹) to the assay buffer (10 mM creatine phosphate, 0.5 mM ATP, 100 $\mu\text{g ml}^{-1}$ creatine phosphokinase, 5 mM MgCl_2 , 1 mM DTT, 50 mM Tris-HCl, pH 7.5). Assays were terminated by addition of SDS⁹. Samples were analysed by electrophoresis on discontinuous 12.5% polyacrylamide-SDS gels. Lane a, from a mock assay (extract omitted); lane b, a complete yeast assay but in the absence of ATP (and presence of 0.6 units of yeast hexokinase and 20 mM 2-deoxyglucose); lane c, an analogous 6 min assay with ^{125}I -ubiquitin and 120 μg of rabbit reticulocyte extract prepared as described previously¹⁰. Lanes d-g, complete yeast assays carried out for 3, 6, 15 and 30 min, respectively. Arrows indicate the electrophoretic origins.

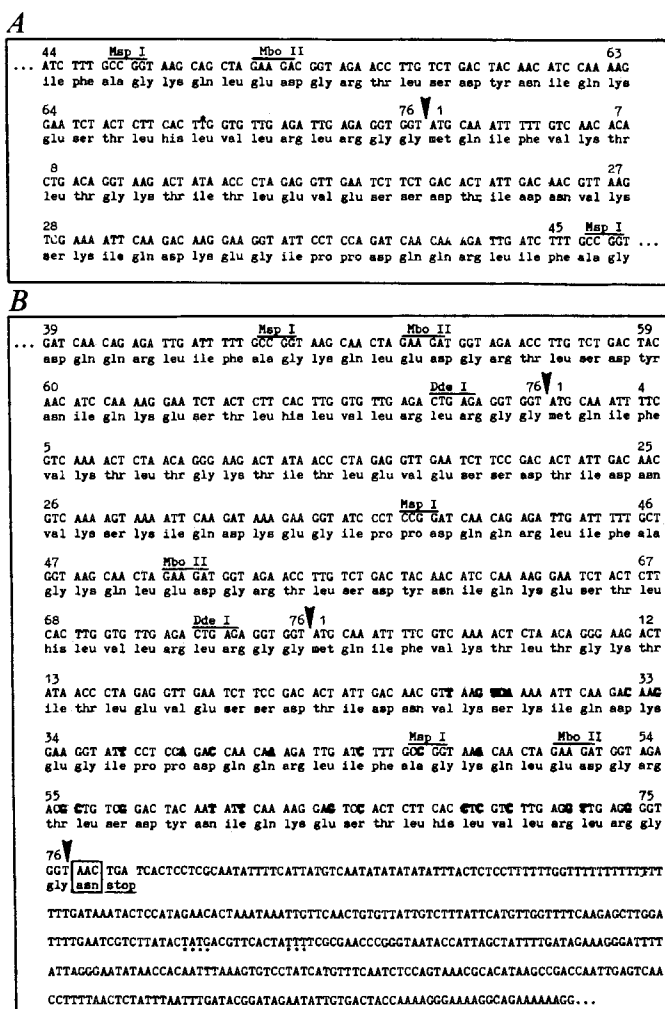
nucleotide sequence homology to the pUB1 insert is restricted in *S. cerevisiae* to a single genomic locus (Fig. 3D).

While the data of Fig. 3, based on restriction fragment analyses, adequately demonstrate the existence of multiple repeats of yeast ubiquitin-coding elements, the precise juxtaposition of these ubiquitin repeats (Fig. 2A) was shown to be general by more extensive nucleotide sequence determination (see Fig. 2B). Since the ubiquitin-coding repeat is of a size (228 bp) appropriate for sequence determination from a single site, the ubiquitin-coding region could be sequenced by placing each repeat next to a universal sequencing primer site, such as that of the mp family of M13-derived cloning vectors¹⁸. The desired M13 subclones correspond to the restriction fragments visualized autoradiographically in Fig. 3B. In a preparative counterpart of the same experiment, DNA was eluted from gel slices corresponding to the autoradiographic bands in Fig. 3B, and the fragments of interest were cloned selectively into the M13mp9 vector¹⁸ (see Fig. 2B legend).

Sequencing of the nested set of ubiquitin-coding repeats, subcloned into M13 as described above, substantiated in detail

Fig. 2 Nucleotide sequences of the yeast ubiquitin gene. Arrowheads indicate sites of proteolytic cleavage required to generate mature ubiquitin from a polyubiquitin precursor; predicted amino acids are thus numbered from 1 to 76 beginning with predicted mature amino termini (see Fig. 4 and text). **A**, Complete nucleotide sequence of pUB1 insert. **B**, nucleotide sequences spanning the 3' end of the yeast ubiquitin gene.

Methods: **A**, The plasmid pUB1 contains a 240 bp insert subcloned from the λ UB phage. λ UB was isolated as described in the text from a yeast genomic DNA library carried in the λ gt11 vector¹³ (for preparation of the library see ref. 14). The polyclonal antibodies against human ubiquitin were affinity-purified on ubiquitin-Sepharose before immunoscreening at a dilution optimized in preliminary dot immunoassays. These antibodies were negative with *E. coli* extracts. Screening of the library (carried out via plaque immunoassay, at 5×10^5 plaques per 15-cm plate, with the host *E. coli* Y1090, as described previously¹⁴) yielded fifty apparently positive phage stocks. Two of four such stocks, tested further, produced immunological signals specifically inhibitable by added ubiquitin (not shown). One such phage, λ UB, was plaque-purified until each visible plaque (of about 250 tested) was immunoreactive. The immunoreactive protein specific to λ UB lysates is ~50 kilodaltons in size, and its expression is IPTG-independent (data not shown). DNA from the cloned λ UB phage was digested with *EcoRI* to yield the 2.4 kb λ UB insert, which was then purified by gel electrophoresis and sheared by sonication to an average size of ~400 bp. The resulting DNA fragments were subcloned into the pUC13 plasmid expression vector¹⁶ using synthetic linkers. The pUC13 library was immunoscreened with the anti-ubiquitin antibody, using previously described methods³¹; one of the positive clones, designated pUB1, was purified and its insert was sequenced on both strands, using the method of Maxam and Gilbert¹⁵. **B**, The insert of λ UB was excised with *EcoRI* and subcloned into the *EcoRI* site of pBR322, to yield the pUB2 plasmid. pUB2 was cut with *HindIII* (see Fig. 3A), and a small portion of the resulting DNA sample was ³²P end-labelled with the Klenow DNA polymerase I fragment³², to serve as a tracer, then added back to the same DNA sample. The resulting mixture of labelled and unlabelled DNA was then cut with *PstI* (see Fig. 3A). The insert-containing fragment was purified by agarose gel electrophoresis, partially digested with *MspI*, treated with bacterial alkaline phosphatase, and analysed on a 3% polyacrylamide gel (see Fig. 3B). The wet gel was subjected to autoradiography, and DNA was eluted from gel slices, each corresponding to an autoradiographic band. The resulting DNA samples were ligated in parallel to the *HindIII* site of replicated form DNA of M13mp9 phage¹⁸. The vector DNA had been previously digested with both *HindIII* and *PstI*, so that only one *HindIII* site was available for ligation. Following heat-inactivation of DNA ligase, the samples were digested with *AccI* to provide a single-stranded end on the vector which is complementary to the *MspI*-generated single-stranded end of the inserted fragment. Ligation products of the desired size were then purified on an agarose gel, recircularized via *AccI*-*MspI* ligation, and transformed into *E. coli* JM101. Phages from plaques which appeared white on plates containing Xgal and IPTG were amplified, and replicative form DNA was prepared and digested with various restriction enzymes to verify its expected identity. Only the expected M13 subclones were obtained using the two-step ligation scheme described above, that is, all subclones had inserts of the same size as their 'parental' restriction fragments, and the subcloned inserts were specifically oriented, with ubiquitin-coding sequences proximal to the universal sequencing primer site. This site was used for sequencing roughly 280 nucleotides into each of the overlapping classes of the M13 subclones; the sequence in Fig. 2B was compiled from four such sets of overlapping sequence data. Phage DNA was sequenced by the chain termination method as previously described³³, using [³⁵S- α]dATP (ref. 34). The 'non-ubiquitin' Asn residue encoded at the end of ubiquitin gene is boxed. Differences between the nucleotide sequences of the two ubiquitin-coding repeats nearest to the end of the coding sequence are indicated in the last repeat by shadowing. Note that all of the sequenced repeats encode identical amino acid sequences. A nucleotide sequence which resembles the consensus sequence for transcription termination in yeast¹⁹ is indicated by dots.



the concept of a polyubiquitin precursor protein (Fig. 2B). The sequenced portion of the ubiquitin gene domain includes three and a half ubiquitin-coding repeats and three head-to-tail repeat junctions, each of which encodes Gly-Met, a putative site of processing, as inferred originally from the sequence of the pUB1 insert (Fig. 2A, B). A nucleotide sequence resembling the consensus sequence for transcription termination in yeast¹⁹ is found 160 bp downstream from the stop codon of the ubiquitin gene (Fig. 2B).

Remarkably, only the last of the sequenced ubiquitin-coding repeats encodes 77 rather than 76 residues, characteristic of mature ubiquitin (Fig. 2B). The extra residue, Asn, is at the end of a putative polyubiquitin precursor protein where it immediately follows the C-terminal Gly residue of mature ubiquitin (Fig. 2B). By blocking the carboxy-terminus of the preceding Gly residue, the 'non-ubiquitin' C-terminal Asn could serve to prevent participation of unprocessed polyubiquitin in reactions of ubiquitin-protein conjugation. The properties of recently characterized ubiquitin-specific hydrolases from mammalian

cells²⁰⁻²² are compatible with their involvement in processing of polyubiquitin via cleavages at Gly-Met and Gly-Asn junctions. Complete sequencing of the ubiquitin gene domain (now under way) should show whether the putative polyubiquitin precursor contains any non-ubiquitin amino acid sequences other than the C-terminal Asn residue (Fig. 2B). Preliminary sequencing information (not shown) strongly suggests that the N-terminus of the putative polyubiquitin is identical to that of mature ubiquitin, and confirms the above estimate of sixfold repetition of ubiquitin-coding elements.

The polypeptide organization of the yeast ubiquitin gene is novel because the polypeptides previously described are all either secretory^{23,24} or viral²⁵ gene products, and contain 'spacer' sequences discarded in processing. Furthermore, with the exception of protein precursors to yeast mating factors, which in the case of α -factor^{26,27} contain four identical repeats of the mature α -factor sequence separated by six to eight residue long spacers, no other known polypeptides are internally repetitive to a comparable extent.

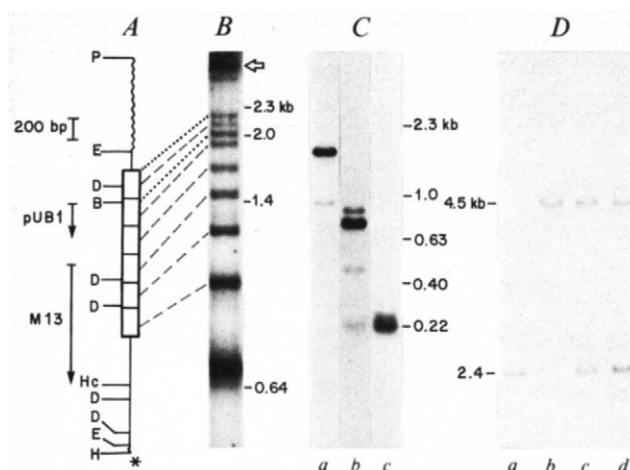


Fig. 3 Structural analysis of yeast ubiquitin-coding sequences. **A**, diagrammatic representation of an insert-containing restriction fragment from the plasmid pUB2 (see text and legend to Fig. 2B) cut with *Pst*I (top) and *Hind*III (bottom). The boxed segment indicates the ubiquitin-coding domain; junctions between ubiquitin-coding repeats are shown as five horizontal lines within the box. These lines correspond to the arrowheads in Fig. 2. The sequenced portions of the insert (see Fig. 2) are indicated at the left by arrows which also denote the predicted direction of ubiquitin gene transcription. Restriction endonuclease sites: P, *Pst*I; E, *Eco*RI; D, *Dde*I; B, *Bgl*II; Hc, *Hinc*II; H, *Hind*III. The asterisk at the bottom right marks the end-labelling site used in panel B (see also Fig. 2B). Wavy lines indicate vector DNA. **B**, periodic distribution of *Msp*I restriction sites in the cloned yeast ubiquitin gene. **C**, structural analysis of ubiquitin-coding sequences in yeast genomic DNA.

Methods: **B**, The plasmid pUB2 (see text and legend to Fig. 2B) was cut with *Hind*III, 32 P-end-labelled with Klenow DNA polymerase I fragment³², then cut with *Pst*I. The insert-containing, end-labelled fragment was purified by agarose gel electrophoresis, partially digested with *Msp*I and analysed by electrophoresis on a 2% agarose gel followed by autoradiography (panel B). Open arrow (top), position of the end-labelled fragment uncut by *Msp*I. This uncut fragment is diagrammed in panel A. The dashed lines between panels A and B point to positions of *Msp*I cleavage sites on the physical map of panel A, and connect these sites to the corresponding radiolabelled fragments seen in panel B. Dotted lines are used to indicate a pair of *Msp*I sites present in addition to the major set of repetitive *Msp*I sites. **C**, Genomic DNA from strain S288C was digested with restriction enzymes, electrophoresed on a 2.0% agarose gel, transferred to Gene Screen (NEN) and hybridized³⁶ using gel-purified insert from pUB1 as a probe. Restriction enzymes: lane a, *Bgl*II and *Hinc*II; lane b, *Dde*I; lane c, *Msp*I. An estimate of six ubiquitin-coding repeats is confirmed by the *Dde*I digest (lane b): fragments at 0.8 and 0.23 kb each contain exactly one repeat (see Figs 2B, 3A), and the prominent fragment at 710 ± 20 bp spans just over 3 repeats, by estimate either of size or relative hybridization intensity, leaving, again by either of these criteria, $\sim \frac{2}{3}$ of a repeat associated with the 0.45 kb fragment (see also Fig. 3A). **D**, ubiquitin-coding yeast DNA sequences are present at a single genomic locus. DNAs purified from the *S. cerevisiae* strain A364A (the same results were obtained with strain S288C) and from the λ UB phage (see Fig. 2) were digested with *Eco*RI, mixed in varying proportions and electrophoresed on a 0.8% agarose gel, followed by Southern hybridization with the purified 32 P-labelled insert of pUB1, which contains exclusively ubiquitin-coding nucleotide sequences (see Fig. 2A). Only the hybridization pattern is shown. Lane a, 8.5 ng (one molar equivalent) *Eco*RI-cut λ UB DNA; lane b, 2.5 μ g (one molar equivalent) *Eco*RI-cut yeast DNA; lane c, 2.5 μ g yeast DNA plus 8.5 ng λ UB DNA (1:1 molar ratio); lane d, 2.5 μ g yeast DNA plus 17 ng λ UB DNA (1:2 molar ratio). Molecular weight markers (not shown) were used to deduce the lengths of ubiquitin gene-specific DNA fragments.

human:	1	met-gln-ile-phe-val-lys-thr-leu-thr-gly-lys-thr-ile-thr-leu-glu-val-glu-pro-	19
yeast:	...	...	...
human:	20	ser-asp-thr-ile-glu-asn-val-lys-ala-lys-ile-gln-asp-lys-glu-gly-ile-pro-pro-	36
yeast:	...	...	...
human:	39	asp-gln-gln-arg-leu-ile-phe-ala-gly-lys-gln-leu-glu-asp-gly-arg-thr-leu-ser-	57
yeast:	...	...	...
human:	56	asp-tyr-aan-ile-gln-lys-glu-ser-thr-leu-his-leu-val-leu-arg-leu-arg-gly-gly-	76
yeast:	...	...	...

Fig. 4 Comparison of the deduced amino acid sequence of yeast ubiquitin with that of ubiquitin in higher eukaryotes. The sequence given for human ubiquitin is identical to that of bovine, trout and insect ubiquitin^{1,3,35}. The amino acids of human ubiquitin are numbered from 1 to 76, beginning with its N-terminus, as defined by amino acid sequence data¹.

Another interesting feature of the ubiquitin-coding repeats is that although the sequenced repeats differ in up to 27 out of 228 bases per repeat (Fig. 2B), they encode identical amino acid sequences (Figs 2 and 4). The predicted amino acid sequence of yeast ubiquitin differs in only 3 of 76 residues from that of ubiquitin in higher eukaryotes (Fig. 4). This striking evolutionary stability exceeds that of histones^{28,29} and other known proteins.

Finally, recent experiments indicate that in mouse ts85 cells, which have a thermolabile ubiquitin activating enzyme⁹, a putative polyubiquitin precursor protein is strongly induced at the nonpermissive temperature (D.F. and A.V., unpublished data). Thus, the apparently unique organization of the yeast ubiquitin gene discovered in the present work may be characteristic also of ubiquitin genes in higher eukaryotes. Although the functional significance of the polyubiquitin gene organization is not yet understood, a number of competing hypotheses may now be addressed directly using the methods of yeast genetics.

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Biotechnology bookshelf

Anita Crafts-Lighty's choice of the best biotechnology reference books published in 1984.

AFTER a flurry of activity over the past two to three years, the rate of appearance of new books dealing with biotechnology seems to have slowed down a little in 1984. This is particularly evident in the reference department. Nevertheless there have still been a few landmark publications which all biotechnologists should know about and few libraries should be without. Those described below are a personal (and far from comprehensive) selection of the best biotechnology reference books published during this year.

Best basics

PUBLIC awareness of biotechnology is growing steadily and at last there is a readable, accurate, up-to-date and balanced introductory account which can be recommended whole-heartedly to one's non-scientist colleagues. It is cheating a little to include Steve Prentis's *Biotechnology — A New Industrial Revolution* (Orbis, London/ George Braziller, New York; £9.99, \$18.50) under the heading of a reference work, but this is an excellent book to turn to for clear, simple explanations of the complex scientific achievements of molecular biology. However Prentis also goes well beyond genetic engineering to look at many other aspects of biotechnology and their impact on society. The reader cannot help being caught up in the author's manifest enthusiasm for his subject matter, but at the same time one can note with relief an absence of hyperbole and very few errors of fact.

Bergey's bugs

FEW microbiologists will not know of *Bergey's Manual of Determinative Bacteriology*, but anyone who has bought a copy in the past few years may have been surprised to notice that the current (eighth) edition was actually published in 1974. *A Shorter Bergey's Manual of Determinative Bacteriology* did come out in 1977 followed, in 1981, by a supplement to the 1966 taxonomists' handbook *Index Bergeyana*, but these were only stop-gaps and a new edition of the basic work has been badly needed for some time. To rectify the situation, Vol.1 of a massive new work, *Bergey's Manual of Systematic Bacteriology*, was published earlier this year (Williams and Wilkins; \$80, £77). John G. Holt is editor-in-chief, but the first volume contains contributions from more than 125 scientists. In over 950 pages, 11 groups of bacteria are covered (spirochaetes, aerobic motile gram-negatives, motile curved gram-negatives, gram-negative aerobic rods, anaerobic rods,

sulphate/sulphur-reducing bacteria, anaerobic gram-negative cocci, rickettsia and chlamydia, mycoplasmas and endosymbionts). Presumably gram-positives, spore-formers and others will be dealt with in Vol.2, which is scheduled to appear late next year. The first volume also has sections on taxonomy and nomenclature, a list of culture collections, an extensive (105-page) bibliography and an index. Its scope is far wider than the determinative manual although much similar material is included. This mammoth tome is not exactly light reading, but the book is unquestionably the most useful and detailed compendium of microbiological information available and a must for every library serving microbiologists.

Boring but better sequences

Nucleotide Sequences 1984 (IRL Press; £50, \$75) must rate as the most boring biotechnology reference book of 1984 to read through; but that is not what it is designed for. This paperback work of 1,221 pages, divided between two volumes, lists over 4,000 reported nucleic-acid sequences containing nearly three million bases. It is the first printed compilation of sequences in the European Molecular Biology Laboratory's Nucleotide Sequence Data Bank (Release 3.0) and the Gen Bank Genetic Sequence Data Bank (Release 17.1). Since the databases are updated periodically, the computer systems will often contain more information than the books, and, of course, these databases can be searched in more sophisticated ways than a printed compilation. But for those without computer access or for the occasional "Has this gene been sequenced?" enquiry, the printed compendium will be of great value. The entire text is reproduced from a computer printout, which leads to admirably concise entries, though there is still room for better presentation in a number of minor ways. For example authors' names should surely be capitalized. Sequences are grouped by source (mammalian, other vertebrates, invertebrates, plants, organelles, bacteria, structural RNA, viruses, bacteriophages, synthetic and so on) and there are author and keyword phrase indexes, plus indexes relating the "entry names" used in the databases to the accession numbers used in the book.

Biotech business

ONE of the most widely quoted biotechnology documents of 1984 is the United States Office of Technology Assessments' report *Commercial Biotechnology — An International Analysis* (US Government

Printing Office \$20). For those concerned with science policy and history, this is an invaluable reference tool, although it contains far fewer handy market figures than the OTA's 1981 report, *The Impacts of Applied Genetics*. Obviously, the publication is aimed primarily at an American audience, and it is therefore somewhat biased in its outlook, but it still represents an excellent in-depth summary of the position of commercial biotechnology worldwide at the beginning of 1984.

British biotech directory

IN the interests of strengthening British biotechnology, the Biotechnology Unit of the Laboratory of the Government Chemist (part of the United Kingdom's Department of Trade and Industry) last year commissioned a directory covering British biotechnology companies and academic institutes. The result is *The 1984 Directory of British Biotechnology* (Cartermill Publishing, PO Box 33, St Andrews, Fife, UK; £48.50). Venture capital firms, contract research organizations, equipment suppliers and engineering contractors are all included, but the coverage of university laboratories and research institutes is somewhat more limited. The entries are accurate and quite up to date, and the book is nicely presented and easy to read. A perfectionist might ask for longer profiles, and a clearer distinction between product producers and contract research organizations, but these are minor failings in what is one of the best reference books of its type.

And the future?

THE ratio of commercially-orientated to scientifically-orientated books in this selection is probably indicative of the needs of biotechnology tomorrow. Looking towards 1985 and beyond, I would like to see more attempts to describe and catalogue the numerous cloned genes, expression vectors and monoclonal antibodies around, and more opinions of the strength of the patents being carefully filed by so many researchers and companies. Some comprehensible books on regulatory affairs, and more down-to-earth advice on the effective management of innovations and on academic liaison techniques, would be welcome too. Downstream processing, scale up and plant genetic engineering are also important areas of biotechnology where scientific advances are speeding up but the reference shelves remain too bare.

Many claim that biotechnology will be the most important industry of the twenty-first century and certainly research progress continues to astound — it should hold an equally bright future for support activities such as publishing, and that will be to the benefit of all concerned. □

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Who does what, and where?

John Galloway

Who's Who in Science in Europe: A Biographical Guide in Science, Technology, Agriculture, and Medicine, 4th Edn.

Longman/Gale Research: 1984. Three volumes, pp.2,556. £295, \$500.

The Medical Research Directory.

Edited by Jean Elizabeth Levy.

Wiley: 1983. Pp.730. £85, \$170.

Medical Research Centres: A World Directory of Organizations and Programmes, 6th Edn.

Consultant editors Leslie T. Morton and Jean F. Hall.

Longman/Gale Research: 1984. Two volumes, pp.1,087. £175, \$320.

World Guide to Scientific Associations and Learned Societies, 4th Edn.

Edited by Barbara Verrel and Helmut Opitz.

K.G. Saur Verlag, Munich and New York (distributed in Britain by Library Association Publishing, 7 Ridgmount Street, London): 1984. Pp.947. DM268, \$112, £80.

SCIENCE, we all appreciate, is *public* knowledge and its most apparent property, other than being quite inscrutable to members of the public and largely disassociated from them, is how much of it there actually is. UNESCO and the International Council of Scientific Unions recognized the problem when in 1971 they published a feasibility study on a World Science Information System. Twenty-two recommendations were made but they did not include registers of scientists or even laboratories. At least not explicitly. But a number of publishers have thought it worth a try.

Where does one start in trying to assess a small sample of such registers? First of all, is it possible to get at the raw numbers of scientific and technological manpower? Well, yes, up to a point. UNESCO's office of statistics produces world-wide figures on these, though the last tables I could get were published in 1980. The total world scientific and engineering manpower comes out at about 33 million, but the sorts of figures here do not simply refer to active research scientists publishing their findings in the open literature. Ominously, Britain doesn't feature in the analysis (I didn't realize things were so bad; perhaps no one could be bothered to fill in the form when it came round).

Suppose you are interested in more than just numbers — you want to know what the quality is like. This is even trickier. You can go for members of the various national academies of science, for example, where membership is by election on grounds of merit — not foolproof of course. After that you are firmly in *Who's Who* land and for the purpose of this review *Who's Who in Science in Europe*. This used to be published by Francis Hodgson but the latest edition comes from Longman. There are 25,000 scientists named (compare this with the UNESCO figure of 4,800,000, excluding Britain), with biographical details on about half of them. The rather similar *American Men and Women of Science* lists 130,000, about

one in twenty of their UNESCO total.

Well, who *is* who in European science? Quite clearly it is the heads of laboratories and of the larger university departments. The compilation started with Longman's own two-volume *European Research Centres* which seems to list about 18,000 government laboratories, departments spending more than £50,000 a year, and some public and private laboratories. This was supplemented by an exercise based upon details of most of the authors of scientific books in the past five years, and also by sending out 50,000 enquiry forms to other senior scientists of whom about a quarter actually replied. Those who did not respond, but for whom some information was available, end up as brief entries. So the two problems of compiling this sort of reference list are revealed — first how to choose who to seek information from, and then to get a decent rate of replies. The consequence is that the quality of the information is rather variable. In the field of neuropharmacology it is in fact misleading — neither Neher or Sakmann (the inventors of patch clamping) figure, Rang's move from the chair of Pharmacology at University College London to be Director of the new Sandoz Institute is not recorded, and Leslie Iversen appears twice, first as L.L. and Director of the MRC's Neuropharmacology Unit in Cambridge and then as Lars Leslie and Director of the new Merck, Sharp and Dohme Neurosciences Institute — someone should have picked that up, even given the long gestation period of such books.

The next question is who consults something like this. Cynically I thought it was the scientists whose names are included. Longman cheerfully admit a cheap offer to those who appear was part of the pitch, but in the end they expect to sell about 1,500 copies to libraries, mostly in Europe but a fair proportion through Gale in the United States. Librarians I spoke to — admittedly not a large sample — would very much like to have it simply as a guide to the more successful and still

relatively active scientists. And for Europe there really isn't anything else. Still, it would be nice to have a *Good Scientists Guide* as well as one for top scientists, that is to say scientists who have achieved administrative positions. This one is really just a guide to personalities. Lack of anything but a rudimentary index prevents it from being anything more.

If you simply want to associate scientists and subjects, the best way into the labyrinth is through the *Science Citation Index* and the *Permuterm Subject Index* which are available as computerized databases. The only biographical database is *American Men and Women of Science*, available through DIALOG; there is nothing for Europe.

So much for who, what about where? If you do want a more detailed picture of science and the scientists doing it then the price that has to be paid is a narrowing of scope — of subject or geography, or both. For the Commonwealth there is the *Commonwealth Universities Yearbook*, in four volumes, a bald listing of the staff members in universities from Australia to Zimbabwe. In Britain the British Library publishes annually the three-volume *Research in British Universities, Polytechnics and Colleges* (RBUPC). The 1984 edition is just out and seems pretty comprehensive with nearly 50,000 scientists mentioned and a detailed breakdown of research by department, broad subject heading and project. (In France this sort of information is available on the Labinfo and CNRS databases.) A further narrowing by subject is apparent in Wiley's *Medical Research Directory* which uses a breakdown very similar to the RBUPC. It features the research of 650 different institutes, using 45 broad subject headings, subdivided first by departments and then by projects. The index contains 17,000 names and 22,000 terms.

In principle you might penetrate British medical research with this volume and identify scientists with particular research interests. On the other hand the breakdown by subject is whimsical. Neuroscience gets only four pages. It includes, correctly, three research divisions at the National Institute for Medical Research but gets only four out of 21 of the MRC neurosciences units. Leslie Iversen features again as Director of the Neurochemical Pharmacology Unit in Cambridge — he resigned some eighteen months ago; and half of the Unit's senior scientists mentioned are no longer there. Dr J.B. Brierley, a neuropathologist stated as being at the MRC Laboratories at Carshalton, has been retired for at least three years. The main flaw, obviously, is that much of what is listed is undeniably out of date. To be quite fair the publishers say they have deliberately included recent as well as current research, but that does not really draw the teeth of the criticism. And given the magnitude of this problem, it is almost

surprising that publishers try to compile such directories at all. What they should do of course — and what in fact Wiley do now do — is to provide the information through on-line databases. These are much easier to keep up to date and can be searched far more effectively than their printed counter parts.

The price paid for broadening the geography, while keeping the general scope, is a loss of detail. Longman's *Medical Research Centres* covers 11,000 laboratories and organizations including charities, commercial laboratories and individual university departments worldwide, arranged alphabetically and by country. A broad idea of the interests of each one can be obtained, quite often together with the yearly budget. Commercial laboratories seem a bit coy about theirs. Again in those areas where I had detailed, up-to-date information, I found these volumes tended to be out-of-date — and sometimes things were not spelt out very well. My favourite entry, other than Dr Iversen's traditional two, was that the Medical Research Council employs 106.5 million graduate staff. The head of personnel is right to worry about over-manning!

My favourite among all these four works, though, is Saur Verlag's *World Guide to Scientific Associations and Learned Societies*, one volume listing 22,000 organizations in all fields, science, technology and the other cultures; very catholic, I could have read it for hours. Here one encounters the American Underground-Space Association (not hollow-earthers, but engineers). And what about the Disinfected Mail Study Circle or the Japanese Correctional Association — it may just refer to the language but I am not sure. This book seemed to me to be truly valuable as a means to cultural insight, but most of the entries are in the language of their country of origin and are usually not translated. Also provided is an 8,000-entry index of the abbreviations for the associations, and then a breakdown by subject, another fascinating compilation. Under humanities, Britain rates only one entry along with Togo, Upper Volta, Iran and Israel, whereas France has 16 and the USSR none.

There is a fair overlap between this volume and the excellent *The World of Learning*, published by Europa, which lists 24,000 universities, museums and learned societies, for instance, and gives a fair amount of detail about each, managing to get in roughly 150,000 of the people associated with them; a new edition for 1984–5 comes out soon. But the strength of Saur's *World Guide* is its breadth — the placing of the large "official" organizations in a proper context of grass-roots societies. Science indeed is more than main-stream institutional research. □

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China: the promise and the problems

Gregory Blue

Science and Technology in China.

By Tong B. Tang.

Longman: 1984. Pp. 269. £45, \$85.

CHINA'S science and technology have developed rapidly since 1949, and in particular since the National Science Policy Conference of March 1978 which aimed at bringing China to advanced international levels by the year 2000. In this contribution to Longman's *Guide to World Science and Technology* series, Tong B. Tang (Deng Tang-bo) presents us with a concise, up-to-date account of the state of play in those fields of applied science and sophisticated-to-intermediate technology designated for priority development at that conference. This volume thus nicely complements the collaborative work, *Science in Contemporary China* edited by Leo A. Orleans (Stanford University Press, 1980), which surveyed the entire range of pure and applied sciences down to the end of the 1970s.

Following a general introductory analysis of science policy, organization and funding, the author proceeds in subsequent chapters to examine agricultural and environmental research, the earth sciences and mineral industries, energy science and technologies, as well as transportation and information technologies. For the chapter devoted to the biomedical sciences valuable contributions have been elicited from Hong Guo-Fan and Jin Neng-Ren, who write respectively about basic research and clinical practice. An interpretive sketch on science and social change in China by John Collier fills in the basic historical background in Chapter 2. The final chapter helpfully (though unusually) gives an *exposé* of the state of science and technology in Taiwan. One might also have liked to see included a similar chapter on Hong Kong (Xianggang, in the *pinyin* rendering of the standard pronunciation); but this is not a serious lack, since knowledge of work done there is already quite accessible.

The author takes due care to identify key institutes, to note which ones have been re-organized in recent years and to describe in some detail the work being done at each. Indication is given of those areas (such as microsurgery, earthquake prediction and satellite tracking and recovery) in which Chinese scientists have already attained advanced world levels. The author also points out that, having started from a very restricted industrial base before 1949, China has recently overtaken Japan as the world's foremost manufacturer of radios; she also now ranks as the fifth-largest producer of steel, although certain high-grade types still remain beyond her

competence. As in the Soviet Union, performance in the pure sciences is generally impressive. Like both the Soviet Union and Japan, however, China faces considerable problems in several areas in moving from basic research through the realm of research and development and into that of economic operationality. And Chinese technological capacity remains low in some crucial economic sectors. For example, in the coal industry 40% of pit faces are still unmechanized; and there are grave shortages in the transportation and energy sectors. Given present priorities, however, such circumstances may well be dramatically changed by the end of the century.

Chinese modernization in the early post-Mao years was predicated on a policy of massive and sometimes ill-considered technology transfer from the industrialized countries. In recent years this policy has been replaced by one of more selective transfer, often under the auspices of agencies of the United Nations. Emphasis seems to have swung towards importing a restricted number of examples of a particular technology and of using native expertise to appropriate it to Chinese conditions.

One supposes that, after a lag of several centuries, the task of bringing Chinese scientific and technological standards back up to international levels in most major fields will require a few generations more. Much progress has already been booked, however; in the light of China's human resources and history, it would be surprising if it were not to continue. At present it would seem ill-considered to keep oneself in the dark about the work of Chinese scientists, for there are few fields in which they have not made significant breakthroughs, as the volume under review shows.

Despite the diversity of its technical subject-matter, the presentation in *Science and Technology in China* is clear, concise and in general easily readable, though perhaps more attention at the sub-editing stage would have been in order. Conscientious internal cross-referencing together with efficient subject — and institutional — indexes make it a useful reference work. □

Gregory Blue is a research associate at the Needham Research Institute, Cambridge, UK, and a collaborator on the Science and Civilisation in China project.

● Other titles published in the *Longman Guide to World Science and Technology* series deal with the Middle East (by Ziauddin Sardar; reviewed in *Nature* 302, 359; 1983), Latin America (compiled by Latin American Newsletters) and, most recently, Japan (by Alun M. Anderson).

Later volumes will cover the United Kingdom, South-East Asia, the Indian Subcontinent, the United States, and Australasia, Antarctica and the Pacific.

Rolling and pitching in Russian

Vera Rich

Russian - English Translators' Dictionary: A Guide to Scientific and Technical Usage, 2nd Edn.

By Mikhail Zimmerman.

Wiley: 1984. Pp. 544. £31, \$59.95.

Dictionary of Scientific and Technical Terminology: English, German, French, Dutch, Russian.

Edited by A.S. Markov *et al.*

Martinus Nijhoff: 1984. Pp. 496.

Dfl. 90, \$34.50, £22.95.

DICTIONARIES, and technical dictionaries in particular, must surely be judged not only on the accuracy and contemporaneity of their contents, but also on how far they serve the needs of their intended users. These two volumes, very different in intention, differ too in their achievement of that aim.

Zimmerman's *Translators' Dictionary* has been a standby for the professional user since its first appearance in 1967. It is not a normal dictionary, listing not so much individual words and concepts, but the constructions linking them and the idiomatic turns of phrase which transform a piece of translationese into a readable text. Constructions are indicated by an example, usually a complete sentence, with the words in question printed in bold. Thus: "OTVERGAT' GIPOTezu: By 1950, the tetranucleotide hypothesis **had been overthrown**, (or **ruled out**, or **rejected**)". This format is particularly helpful in coping with those Russian words which have a number of near-synonymous renderings. No less than seven examples are listed for "vrashchat'sya" in its various meanings of to rotate and to revolve, while for the derived phrase "vrashchat'sya vokrug osi" we have this splendid example: "Like an airplane, an insect can **roll around its longitudinal axis**, **pitch around a horizontal axis** perpendicular to its direction of flight, or **yaw around a vertical axis**".

A dictionary of this type cannot be employed without a good special-subject dictionary for the text in hand. In combination with such a work, however, Zimmerman's *Translators' Dictionary* has been proving its worth for almost 20 years. This new, updated edition will come as a boon to the younger generation of translators who, until now, have been obliged to work with library copies unless fortunate enough to inherit a copy from someone about to retire from translation.

In compiling their book, Markov and his colleagues have attempted to produce a "general purpose" dictionary, based on the vocabulary "pertaining to general study courses ... given in technical colleges irrespective of their specification".

Revealing as this is of the broad nature of such courses in Russian colleges (opening at random, one observes, pp. 62-63, that students have to be conversant with such varied concepts as cloud-chambers, co-factors, cold-working and collapsars), the result is a somewhat confusing conglomeration. Too many concepts are included for the book to be of more than partial use to anyone but a professional translator, who would, presumably, prefer a specialized dictionary for each topic.

A more serious complication stems from the fact that English is chosen as the main language (with cross-references in the other languages). This raises the difficulty of the differences between British and American spelling, which has here resulted in some odd inconsistencies. Both "center" and "centre" are listed, "center/centre of gravity" occurs with both spellings, but "center rest" and "centre of curvature" occur only once. Moreover, "center" is rendered as "centrum" in Dutch, while "centre" can be either "centrum" or "middelpunt"; and although "center of gravity" is "gravitatiecentrum", "centre of gravity" becomes, for some reason, "zwaartepunt". The compilers also have

a somewhat archaic view of English — was it really necessary to list "quicksilver"? — and seem to have a marked reluctance to list an adjective without a noun; thus, the adjective "mathematical" occurs six times, grouped with "expectation", "induction", "logic", "model", "pendulum" and "programming", although all the qualified nouns occur elsewhere (in some form) in the *Dictionary* and all are rendered by a straightforward noun + adjective construction (save for the German *Erwartungswert* which is allowed to drop its adjective).

The listing of complex phrases, though essential in a work such as Zimmerman's intended for the translator who has to pay attention to style, is unnecessarily cumbersome in a work meant for "scientists and engineers" whose need primarily is to read and understand professional material. It is particularly awkward in this book where the five-language format has led to crowding and illegibility which is further exacerbated by the generally cramped and unattractive layout. □

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Words, words, words

Michael Spencer

Concise Science Dictionary.

Edited by Alan Isaacs, John Daintith and Elizabeth Martin.

Oxford University Press: 1984. Pp. 758.

£12.95, \$22.50.

THE world's best-known lexicographer, in defining the word "dull" for his *Dictionary of the English Language*, gave as an example "To make dictionaries is dull work". One test of a dictionary is whether this shows through in the reading of it: would anyone choose to read it for pleasure and education, or only as a last resort when baffled and irritated by an unfamiliar word?

Much depends on the enthusiasm of the compiler, and it is probably inevitable that a committee job such as the present volume will be less fascinating (though more reliable) than the idiosyncratic brainchild of a single author. The editors have, however, achieved their well-defined aim of providing a handy and readable book of reference for school and first-year university students, and for laymen who want to know the meaning of the commoner scientific terms. A *Nature* reader bemused by a title such as "Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene" will at least be able to look up "transcription" and "oncogene", plus about one word in ten of the text of the article itself.

There are over 7,000 definitions (which could, incidentally, have been augmented

by omitting the spaces between entries); line drawings and tables are included where necessary, and there are useful appendices on units, fundamental constants and so on. One passes from "bark" to the "Barkhausen effect", and from "plate tectonics" to "platinum". The content is severely practical, so that although the quark is defined one looks in vain for the derivation of this intriguing word (it was, in fact, lifted from James Joyce's *Finnegans Wake*). The philosophy of science is not covered, so that Occam's razor and other concepts of incalculable value to the budding scientist are not mentioned; reduction in the chemical sense is defined, but reductionism is not.

It is interesting to note how some ordinary English words are used by specialists in different disciplines for quite different purposes: "accommodation", for instance, has three distinct definitions in animal physiology, botany and animal behaviour. One may quibble about the relative emphasis given to different entries — a dictionary offers unlimited scope for quibbling — but this probably again reflects the multi-author genesis of the work. While Ohm's law clearly deserves the paragraph devoted to it, one may question whether the ohmmeter (a meter with which you measure ohms) warrants an entry of the same length. On the whole, though, I think the book will successfully fill an obvious gap in the market, and will find its way into the Christmas stockings of young scientists of ages nine to ninety. □

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Pups and groundhogs

Alan Hendry

Dictionary of Ceramic Science and Engineering.

By Loran S. O'Bannon.

Plenum: 1984. Pp. 303. \$45, £42.75.

"CERAMIC science and engineering is one of the oldest yet one of the newest, fastest growing ... of man's endeavours". Dr O'Bannon begins his preface to the *Dictionary of Ceramic Science and Engineering* with this comment, and therein lies the problem for the etymologist. Ancient origins and the speed with which ceramic science is expanding and incorporating terms from associated disciplines make it impossible to provide a definitive compilation.

Dr O'Bannon recognizes these problems, and based on his wide experience of the subject he has made a brave attempt at providing a reference book to suit several tastes. In so doing, however, he has fallen between several stools. The *Dictionary* is not detailed enough for the professional ceramist and yet is too detailed for non-ceramists. Extensive sections are devoted

to rare earth compounds but the data are of limited value. The information on applications is useful but the representation used in giving chemical data is misleading; for example, the "silicon carbide composite" $\text{SiC} \cdot \text{Si}_3\text{N}_4$, molecular weight 180.28, would be taken to imply a chemical compound of one molecule SiC and one molecule Si_3N_4 , but which does not in fact exist.

The book is dedicated to promoting the standardization of terms in ceramic science and engineering and any attempt in this direction, particularly in a trans-Atlantic context, is to be commended. The bibliography provides a useful source from which to follow up the information contained in the text. Dr Johnson remarked that "making dictionaries is dull work", but omitted to mention that when made they are useful; in both senses this volume is no exception. Readers will no doubt find definitions with which they disagree, but sufficient reason for buying the book is that there is no better alternative available. □

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Business in science

W.F. Clocksin

Dictionary of Computers, Data Processing and Telecommunications.

By Jerry M. Rosenberg.

Wiley: 1984. Pp. 614. Hbk £29.95, \$29.95; pbk £14.95, \$14.95.

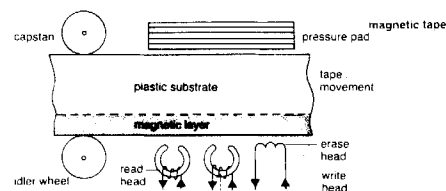
Dictionary of Computing.

Oxford University Press: 1984. Pp. 393. £15, \$25.

AT ONE and the same time computing is a thriving academic subject, a growth industry as well as a new hobby for many. Computer science, in which the fundamental principles of computation are studied, is a subject area of prime interest in universities and research centres. There has been explosive growth in the applications of computing to business problems, including commercial data processing, telecommunications and the technology of information handling. Such a rapidly expanding and diverse field calls for up-to-date reference books which bring together the principles with the practice.

The large *Dictionary of Computers, Data Processing and Telecommunications* is only partly a success. Containing more than 10,000 entries, it is directed mainly towards the commercial applications. Many entries are in fact extracts from other dictionaries and technical standards documents, while others are a mixture of marketing slogans and singular neologisms; I am uncertain whether it is justifiable

to confer respectability on such terms by documenting them in this way. Typographical errors are in evidence, and there are some spectacular blunders, such as equating the "holes" in semiconductor materials with the holes punched in paper cards. Together with other examples, this rein-



Picture "magnetic tape" and "read head"! (Reproduced from the *Cambridge Illustrated Thesaurus of Computer Science*, by Arthur Godman, published by Cambridge University Press price £4.95.)

forces my impression that the author does not know the territory well.

I prefer the *Dictionary of Computing*. With about 3,800 well-chosen entries it is much smaller than the previous volume, but it is superbly edited, with instructive entries and intelligent cross-referencing. Terms in computer science are strongly represented, but the commercial world of data processing is not ignored. Unlike Rosenberg's book, some entries include useful drawings and tables. This is a valuable and reliable reference for the student as well as the computing professional.

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In terms of computer graphics

Julian Gallop

Dictionary of Computer Graphics.

By John Vince.

Frances Pinter, London/Knowledge Industries Publishing, White Plains, New York: 1984. Pp. 132. £15, \$34.95.

ONE might expect a dictionary on a technical topic to contain rather formal definitions of a number of terms. In *Dictionary of Computer Graphics* John Vince does indeed follow the structure of alphabetically ordered entries, but he has chosen to unite them in a descriptive manner with generous use of figures. This gives a good introduction to the chosen topics, even if a few entries are rather longer than is usual in a dictionary. There is a slight danger in the simplicity that this encourages; for example, a statement made under "hidden-line removal" about a regular polyhedron can in fact be made with more generality about a convex polyhedron and would give more insight into the reasons for the statement. However the overall style does encourage the reader to explore the book.

My reservations are mainly about the organization of the *Dictionary* and the selection of terms for inclusion. There are cases where the specific can be consulted, but not the general; for instance one can look up Bézier curves (and patches) but not discover that B-spline is an alternative technique described in the book — if curve-fitting were an entry it could refer to both. In a field where terminology has not stabilized, and several terms are used for similar concepts, a dictionary has a duty to record them, because the reader will come across those terms elsewhere; thus picture plane is defined in the context of two-dimensional projections of three-dimensional scenes, but not projection plane or view plane, which are used in a well-regarded textbook.

Some terms used internally are not defined, while others are defined as a group; thus PAL, the method of encoding television signals used in Britain is described but cannot be looked up because it appears under NTSC which is the American system. The person learning to program some computer graphics applications will not find any mention of such commonly accepted terms as segment, viewport or pick. Possibly the book is not intended for such people.

The *Dictionary* could, then, have been more thorough and balanced. But it is attractively written and presented, and conveys an infectious enthusiasm for the subject. □

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Learning of the gods

P.W. Atkins

McGraw-Hill Encyclopedia of Chemistry.
Editor-in-chief Sybil P. Parker.

McGraw-Hill: 1984. Pp. 1,195. \$76.50, £48.50.

Van Nostrand Reinhold Encyclopedia of Chemistry, 4th Edn.

Editor-in-chief Douglas M. Considine.
Van Nostrand Reinhold: 1984. Pp. 1,082.
\$89.50, £95.

HERMES Trismegistos, the Egyptian chemist (and god), was reputed to know everything and to have written it all down in 36,525 volumes. Latter-day Hermes must contend with publishers, pockets and profits, and must confine themselves to a 1,000 pages or so. But given that these two hefty volumes do summarize the present state of chemistry (a question we return to later), what impression would we take away from them about the range of the subject and its present achievements?

First, a few facts. The McGraw-Hill version (henceforth Hermes-M), cuts chemistry into about 800 pieces. The van Nostrand version (Hermes-N), slices more finely, and ends up with around 1,300. Hermes-M is extracted from the fifth edition of the *McGraw-Hill Encyclopedia of Science and Technology* (1982). Hermes-N claims to be fully revised and much enlarged from the earlier editions, with about 90% of the material being new. Both Hermes wear the hats of specialist contributors from around the world; but while M ascribes every entry to its author, N is much more coy and many pages sometimes pass before a name appears. I very much prefer M's approach in this respect: it is helpful to see an attitude subscribed by authority, especially if it conflicts with a prejudice. Moreover, if a point seems questionable, it is useful to know that the apparent cross-support from another entry is written by another hand.

Quite different impressions of chemistry are obtained at first glance. Hermes-N is conventional in appearance with a somewhat muddy page; the line drawings are amateurish and poorly labelled. Hermes-M, on the other hand, is much more striking in appearance, with a page that looks inviting, neatly tinted boxes displaying structures, and well-drawn illustrations. Both have quite good, but not perfect indexes, which are essential in works as big as these. Here N wins hands-

down, with around twice as many entries as M. The smaller count reflects M's lower density of cross-referencing; for example, "orbital" does not appear as such in M, but has to be sought under "molecular orbital", which is not quite helpful enough, at least to a non-specialist.

It is once we move beyond first impressions that we get strikingly different visions of the present state of chemistry. From N it seems that a chemist is one who knows about sphene, spinel, spodumene and spray drying (to take a page at random). A page more typical of M contains second-order transition and SIMS. Hermes-M knows not of spodumene and spinel. Hermes-N is free of SIMS.

Hermes-N is the traditional fellow, the god of foremen, plant managers, geologists and agriculturalists. N is largely the god of the technological chemist (who might understand his old-fashioned units). While N gives some helpful synoptic surveys of large fields (antibiotics for example), which are missing from M, there

are some bizarre judgements. Why no mention, for instance, of NMR, probably the single most important physical technique available to chemists? Why six pages with do-it-yourself style diagrams on the fabrication of optical fibres?

Hermes-M, on the other hand, is a much more academic god. He seems to know little of traditional chemistry but a lot about spectroscopy, vinylology and oscillating reactions. No nut-shell carbon for him.

For information about traditional chemistry, and particularly chemistry with a technological tinge, then Hermes-N is appropriate but a bit old-fashioned. For information about modern aspects of chemistry, with an up-to-date view of its current achievements, then Hermes-M is undoubtedly the better source (and, at the price, the better value). □

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Eminent lives

Christopher Spencer

Biographical Dictionary of Psychology.

By Leonard Zusne.

Aldwych Press, 3 Henrietta St, London:
1984. Pp.563. £47.50, \$49.95.

ABRAHAM, Karl (German psychoanalyst) to Zwaardemaker, Hendrick (Dutch otolaryngologist), but this is far from being just a dictionary of psychologists. Zusne has provided an excellent source of reference to the intellectual history of psychology: 544 brief biographies which, taken together, trace the development of ideas from Thales, Pythagoras and Heraclites, through the mediaeval scholastics and Arab philosophers, to the origins of modern science and the emergence of psychology as a distinct discipline.

Each entry has a common format: place and exact dates of birth and death; highest degree awarded; positions held; then several paragraphs describing and evaluating the work of the person concerned and its significance for psychology; and finally a bibliography of fuller biographies of the individual.

One can thus use the book as an historical encyclopaedia or browse with profit through the excellently-summarized biographies: Zusne shows that brief entries can be succinct without trivializing the subject matter. The volume will serve well as a sourcebook for amateurs in the history of science, especially those interested in the cross-influences between disciplines, and includes an appendix which lists the nineteenth- and twentieth-century contributors to psychology by their principal university or institution. This appendix dramatically illustrates the Germanic domination of

nineteenth-century science, and the shift to American pre-eminence by the early twentieth century. Indeed, many individual biographical entries show that there was a movement of persons as well as ideas: Jastrow, Rapoport, Razran, Reich, Spitz and many others were born and educated in central and eastern Europe, but spent much of their working life in the United States.

Another appendix is perhaps of lesser value: Zusne takes up 16 pages with a table of "relative eminence", a ranking of all the contributors to psychology according to a count of pages devoted to each individual in 16 histories of psychology. Thus, in this table, Maslow beats by a short head St Albertus Magnus, who in turn ranks one place ahead of Kraepelin.

The only serious limitation of the book stems from Zusne's understandable reluctance to adjudicate between the relative claims of the living for inclusion in the *Dictionary*, and his consequent limitation of entries to the distinguished deceased. This imposes a curious selectivity of coverage of the subject. Thus, for example, Berlyne, Olds and Lenneberg, born in the 1920s, are eligible for inclusion because of their relatively early deaths; whereas many equally eminent figures of twentieth-century psychology are still happily living, and thus ineligible.

This limitation on one side, the *Dictionary* is a welcome reference work. One final thought for our own over-productive age that remains with me, after several pleasurable hours of browsing, is that many of these biographies demonstrate that impact does not necessarily require a large output. □

Christopher Spencer is a Lecturer in the Department of Psychology at the University of Sheffield.

● Also newly published by McGraw-Hill is the *McGraw-Hill Dictionary of Chemistry*, containing terms and definitions selected from the third (1984) edition of their *Dictionary of Scientific and Technical Terms*. The chemical dictionary includes more than 9000 terms with emphasis on the vocabulary of theoretical and applied chemistry, and costs \$44.95, £28.50.

Pieces of mind: more for the clinician than the academic

Stuart Sutherland

Longman Dictionary of Psychology and Psychiatry.

Edited by Robert M. Goldenson and Walter D. Glanze.
Longman: 1984. Pp. 816.
£29.50, \$39.95.

THERE is a real need for an up-to-date dictionary of psychology; as far as I know, none has been published since 1978. Despite the fanfare in the blurb ("21,164 entries or headwords, twice to five times the number of other dictionaries in these fields"), the *Longman Dictionary of Psychology and Psychiatry* fails to fill this gap.

The psychological entries contain many errors and omissions. The latter include many terms in common use, such as "two-process learning theory", "episodic memory", "iconic storage" and — less common, but still important — "anorthoscope". Students frequently confuse "focussing" with "fixating", but this book will not help them: "fixating" is not the "focussing of both eyes on a single object", it is positioning the eyes in such a way that the same object falls at the centre of each fovea (the same error recurs elsewhere in the *Dictionary*). The definition of "receptive field" would have been correct fifty years ago, but does not capture the way the expression is most often used today. Many Pavlovian terms are too loosely defined — "Pavlovian inhibition" is not the "suppression of a response", it is a *process* that may lead to the suppression of a response; the unconditioned stimulus follows the conditioned one, it is not merely paired with it and so on.

A half-hearted attempt is made to cover some of the fields that are now relevant to psychology, for example linguistics, but oddly there appear to be almost no terms from computing science or artificial intelligence: the definition of "heuristic" is grossly misleading since its technical sense is omitted.

The *Dictionary* appears to do better with psychiatric terms, of which it includes many more than the latest edition of Oxford University Press's *Psychiatric Dictionary* (1981); hence, it may be of more use to psychiatrists than to psychologists. But when confronted with this weighty volume, members of both professions may well wish that progress in their respective fields had been commensurate with the amount of jargon they have generated. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

Environmental issues

Timothy O'Riordan

Dictionary of the Environment, 2nd Edn.

By Michael Allaby.
Macmillan, London: 1984. Pp.529. Hbk £25; pbk £7.95.

Directory for the Environment: Organisations in Britain and Ireland 1984-5.

Edited by Michael J.C. Barker.
Routledge & Kegan Paul: 1984. Pp.281.
Pbk £8.95, \$19.95.

PRODUCING a dictionary must be a labour of love. The intellectual rewards are relatively small and the scope for criticism over omissions or nuances of meaning is considerable. Michael Allaby's second edition of *Dictionary of the Environment* comes six years after his first. The contents are much fuller and he has benefitted from the advice of a small company of distinguished friends. The entries are mostly very short, covering clearly and competently all the basic physical, chemical, biological and geographical phenomena. The book's conciseness is a great asset: some 6,000 entries are crowded into 529 pages. However some entries are superfluous — or at least appear so because of the apparent determination to be brief. For example "Environment, Department of" is described as "Department of the Environment" — no mention as to country, or function or date of birth. Similarly EPA is merely described as "Environmental Protection Agency". This is hardly helpful, and a relatively modest expansion of information would have paid dividends.

There are also some curious omissions. There is no entry for environmentalism or environmentalist, nor is there anything under acid rain or acid deposition. There is an entry under environmental quality standards (an American term now embraced by the European Commission), but nothing for environmental quality objectives, a concept much protected by British regulatory authorities. In similar vein, the entry under "best practicable means" only connects it to air pollution control when it now has much wider application. The notorious phrase "as low as reasonably achievable", now in such dispute in the wake of events at Sellafield, does not appear at all. This is a dictionary of specifics rather than concepts.

Michael Barker's *Directory* is altogether a more worthy book, even though it was probably easier to collate than Allaby's *Dictionary*. Barker has sought to compile a list of all official and voluntary organizations which have something to do with environmental management and environmental protection. He appears to have sought his information with care but within a limited budget: inevitably some of the entries are piecemeal and inaccurate. As this is to be the first of a series, greater

comprehensiveness and accuracy should be attained later on.

The entries are listed in alphabetical order. Each provides a name, address, contact phone number and a brief description of activities together with a list of relevant publications. There is a good subject index which allows the reader to look up the variety of organizations interested in a particular theme. For example there are 34 entries under "energy conservation" and 67 under "nuclear power". Maybe next time Barker will delve into the *Civil Service Yearbook* to seek out the sub-compartments of government departments responsible for particular aspects of environmental matters.

Nevertheless this is a valiant and valuable production. Barker has uncovered all kinds of interesting groups. What about HOOVES, which aims to make roads a safer place for horses and to encourage the provision of livery stables and "parking" places for horses in cities? Or Green Deserts, which promotes and participates in desert reclamation schemes and seeks to bring the facts about desert reclamation to the attention of the general public? Students and researchers should take note: many of the organizations listed provide information upon request and they are often well qualified and motivated to do so. Here is an excellent reference book. □

Timothy O'Riordan is Professor of Environmental Sciences at the University of East Anglia.

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Green and pleasant

Richard Mabey

The Macmillan Guide to Britain's Nature Reserves.

Edited by Robert Boote.

Macmillan, London: 1984. Pp. 717. £30.

RSPB Nature Reserves.

Edited by Nicholas Hammond.

RSPB/Croom Helm: 1984. Pp. 189. £9.95.

WITH the centenary of the first nature reserve (Breydon Water in Norfolk) and the 900th anniversary of the Domesday Book both only a few years away, it is an apt moment to produce an inventory of Britain's protected acres. Macmillan have sidestepped ideological wrangles about whether reserves exist principally for wildlife or for the human beings who created them by publishing a monumental guide that is both reverent of its raw material and thoroughly populist in approach. The basis for inclusion of a site, it states, "is that members of the public should have access either by common law rights, or by membership of a club or trust". There is easy public access inside the book, too, thanks to a clear and forthright design, simple, foolproof keys (with map reference, acreage, ownership, best time of the year to visit) and vivid descriptive accounts of the natural features of each site that on the whole avoid scientific jargon.

And what a feast it all adds up to! Seven hundred pages containing entries on some 2,000 reserves, ranging across Britain from Kyemel Crease in Cornwall, a tiny patch of scrub woodland developed over 121 abandoned cliff-top fields, to the puffin-stacked cliffs of Hermaness National Nature Reserve in Shetland. Every imaginable kind of sanctuary is featured: flooded brickpits, old commons, municipal country parks, water authority reservoirs, the occasional free-enterprise

private wood and whole National Parks. The potential users of the book are just as various. For apprentice twitchers it will be an invaluable crib, thanks to its species index; for holidaymakers it would work as a rewarding alternative tourist guide. (Try it out on unpromising Notts, and read about the splendid gravel pits called Attenborough!)

It would be possible to see this book, only slightly cynically, as *The Good Habitat Guide*, and have a melancholy vision in which spontaneous encounters with nature are superseded by something approaching reserve-crawling. At £30 a copy though, I rather doubt it. In my local library they have placed it on the table between the tourist leaflets and the telephone directories, and that seems to me an accurate and rather complimentary assessment of its social role.

Those who prefer their guidebooks to provide ecological background material as well as information, can turn to the Royal Society for the Protection of Birds' account of its own holdings. There are less reserves covered here (some 90, occupying over 100,000 acres) and the book is oddly evasive about access arrangements, but as a bonus there are a number of concise and thoughtful essays on the history and management of different kinds of reserve habitats.

Although I personally find the necessity for nature reserves a sad commentary on our mistreatment of the natural world, I warmed to these two books. It is, after all, better that the last oases are preserved than not. And it would be hard not to be lifted by the images that shine through both guides — of natural landscapes the immense variety of which can still be experienced, and of the people whose hard work and devout attentions have saved their remaining fragments. □

Richard Mabey is a member of the Council of the Nature Conservancy Council, and a writer on countryside matters and landscape history.

Poisoned principles

J. D. Phillipson

Poisonous Plants in Britain and Their Effects on Animals and Man.

By Marion R. Cooper and Anthony W. Johnson.

Her Majesty's Stationery Office: 1984. Pp. 305. £12.95.

A Colour Atlas of Poisonous Plants.

By Dietrich Frohne and Hans Jürgen Pfänder. Translated by Norman Grainger Bisset.

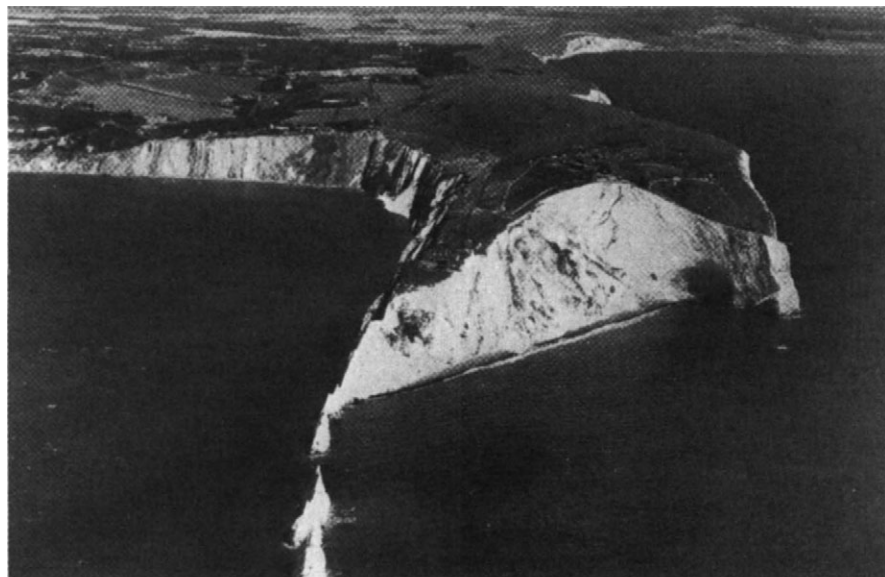
Wolfe Scientific, London/Sheridan House, White Plains, New York: 1984. Pp. 291. £30, \$38.

WHEN two books on the same topic emerge at about the same time, it might be thought that the reviewer should be able to assess which one is the better buy. In this case it is not possible because these two books in fact complement one another rather than compete.

The MAF Bulletin 161, *British Poisonous Plants*, was last reprinted in 1979 and was in need of a complete revision. M. R. Cooper and A. W. Johnson have done just that, bringing together the botanical and veterinarian aspects of poisonous plants. The most common cases of animal poisoning are due to bracken, ragwort, brassica, yew, oak and the umbelliferous plants. Treatment is briefly discussed with the sensible caveat that "the doctor and veterinary surgeon... should be consulted immediately". A section on poisonous principles describes various alkaloids, glycosides, nitrites, oxalates, photosensitizers, proteins, peptides, amino acids and tannins, but no structural formulae are provided for the chemically minded reader.

The book is then divided into sections on fungi, pteridophytes and the flowering plants, the species being listed within their families which are in alphabetical order instead of the former taxonomic order. Over half of the book is devoted to the families, ranging from Alliaceae to Umbelliferae, and each plant is discussed under a series of headings such as poisonous principles, poisoning in animals, human poisoning and treatment. There are some colour prints but these are relatively few, the first one not appearing until p.94, and the photographs are of plants in their natural habitat so that they are not necessarily easily recognized. Each section is supplied with a liberal number of references and there is a good general index.

How does the volume by Frohne and Pfänder differ? First, and most obviously, it is beautifully presented with much better, clear colour photographs usually taken against a light background. Each plant, again listed in alphabetical order of families, is illustrated, and in many cases there is a photomicrograph or microscopical drawing of diagnostic features



From *The Macmillan Guide to Britain's Nature Reserves*.

Remnant beauty — Tennyson Down and the first of the Needles, off Britain's southern coast.

which aid identification. Structural formulae abound and there is a clearly marked section on treatment of poisoning for each plant.

This volume is a practical one, designed to help quick and easy identification of poisonous plants and, except for one page, is concerned with human not animal poisoning. Fungi are considered to be outside the scope of the book but a number of ornamental "non-British" plants such as *Dieffenbachia* are included, an important practical addition.

Should we be concerned about poisonous plants when only two deaths have been reported between 1962 and 1978 in Britain? Clearly the answer must be yes

because 20,000 enquiries were made at the National Poisons Information Service centres, and there were up to 60,000 plant-poisoning cases of children in Germany. Furthermore, there is not just concern over yew and laburnum but also increased public interest in herbal medicines. Both books take careful stock of such medical plants as comfrey and mistletoe, making balanced judgements on the pros and cons of taking such medication, and are, overall, two lovely productions which were a pleasure to review. □

J.D. Phillipson is Professor of Pharmacognosy at The School of Pharmacy, University of London.

Talk of plants

C.A. Stace

The Penguin Dictionary of Botany.

Edited by Stephen Blackmore and Elizabeth Toothill.

Penguin: 1984. Pp. 390. Hbk £12.95; pbk £3.95. In the United States *The Facts on File Dictionary of Botany (Facts on File, hbk \$21.95).*

Longman Illustrated Dictionary of Botany.

By Andrew Sugden.

Longman: 1984. Pp. 192. £3.95, \$7.95.

SPECIALIZED jargon is one of the more daunting obstacles to the understanding of a technical subject, and that of plant biology poses particularly severe problems — many separate disciplines are involved, and the subject has been accumulating technical terms for over 2,000 years. There would be little use to working scientists or students for an encyclopaedic listing of every term that has ever been coined. Hence the main difficulty for the compilers of desk-top dictionaries is to find a middle way; their products must be small enough to be manageable yet large enough to be informative. These two books both strike the right balance.

The Penguin Dictionary of Botany (hereafter Penguin) contains "over 3,000" entries, compiled by ten contributors under a general and a consultant editor, while Longman's offering has "over 1,200" compiled by a single author. The audience for the two books differs, but with some overlap. Penguin is quite sophisticated and will appeal widely to sixth formers, undergraduates (especially) and to the professional plant biologist. Longman is more elementary and will find its greatest use at the school-university interface.

The main feature of Longman is the large number of colour illustrations (averaging more than one per page), and the grouping of terms into 24 subject sections, necessitating a separate index at the end of the book. Penguin, by contrast, is entirely alphabetical and carries relatively few

illustrations, most of which are charts and formulae, and some of which are rather unnecessary (over half a page for chlorophyll lamellae, for example). Important entries are given a more extended treatment; there is, for instance, almost an entire page devoted to the chloroplast, excluding the illustration. In each book the cross-referencing is excellent.

Both the dictionaries seek to cover more or less the whole range of "botany", including bacteria and viruses, except that agriculture and horticulture as well as plant biotechnology seem to be omitted by Longman. The latter scores, however, by its inclusion of useful appendices on Greek and Latin prefixes and SI units. Both books would have profited from more appendices on topics such as the periodic table, a system of classification and so on.

Criticisms of omissions must be very subjective, but Longman seems to include rather too much classical terminology (e.g. polyadelphous) at the expense of more modern aspects (e.g. plasmid). Errors of fact, however, are less disputable, and I was disappointed to see a major error perpetrated on p. 47 of Longman. Here crossing over is shown (unequivocally in multicolour) to occur at the position of a chiasma, that is genetic crossing over is said not to occur until after chiasma formation, whereas the reverse has been known to be the case for over half a century. Such misinformation is potentially harmful in an often-misunderstood though essentially elementary topic. Seeking reassurance, I turned to the corresponding entries in Penguin, and found, by implication, the same mistake. These are certainly points requiring urgent rectification at the first opportunity.

How successful these dictionaries will prove will become clear only after trial periods with the intended users. But my general impression is that much thought and attention have gone into both, and that both should (and deserve to) become well used. □

C.A. Stace is Reader in Plant Taxonomy and Head of the Department of Botany at the University of Leicester.

Order in mammals

Malcolm C. McKenna

Orders and Families of Recent Mammals of the World.

Edited by S. Anderson and J. K. Jones, Jr. Wiley: 1984. Pp. 686. £63.55, \$54.95.

CHARACTERIZING and keeping track of the taxonomic hierarchy of thousands of genera of mammals distributed among about 30 orders is no easy task, as a succession of classifiers since Linnaeus has found out. In 1967 the editors of the present volume published *Recent Mammals of the World*; now they have updated and retitled it. As before, about 20 highly qualified contributors, some inherited from the first project, cover all living mammalian orders and the constituent families in a reasonably uniform style but with a range of originality.

Each still-living order is given a diagnosis, followed by short synopses of current distribution, geological range and a list of constituent families; there is also a section of remarks in each case. Then the format is followed again at the family level. Unfortunately many of the diagnoses contain characters that are merely descriptive, not diagnostic, but they are still useful.

A separate, well-written and generally succinct account is given of fossil mammals, including groups without living members. The known diversity of extinct mammals is increasing rapidly, mainly through the efforts of palaeontologists using screen-washing methods. This is especially true of small rodents and insectivores, but also applies to other orders. Since the 1967 compilation the number of known fossil genera of rodents alone has doubled, surpassing for the first time the number of living genera of rodents.

Among the accounts of living mammals, five chapters on rodents, one on bats, and one on monotremes and marsupials are outstanding, reflecting the enormous amount of recent work in those areas of mammalogy. The original scholarship displayed means that purchase of the book will be a necessity for many palaeontologists.

The illustrations consist mainly of distribution maps, but a number of figures of animals show enough anatomical detail to be helpful in identification (although some have been reduced excessively). Most of the anatomical illustrations are of the skeleton or skull, but dental patterns are not emphasized. An especially valuable feature of the book is the bibliography; the references occupy almost a hundred pages, and many of them are new since the 1967 edition. □

Malcolm C. McKenna is Frick Curator in the Department of Vertebrate Paleontology, American Museum of Natural History, New York.

Primate profiles

R.D. Martin

Primates of the World: Distribution, Abundance, and Conservation.

By Jaclyn H. Wolfheim.

University of Washington Press/
Harwood Academic: 1984. Pp. 832.
\$46 (US), \$72 (Britain and Europe).

WITH the exception of the *Red Data Book (Mammalia)*, a publication only partly devoted to primates and limited to the most basic information, there is no standard reference work regarding the conservation status of primates. In *Primates of the World*, Jaclyn Wolfheim has set out to remedy that situation.

In the book, a vast amount of literature is covered (there are over 1,000 references) and the available information is brought together into a well-designed framework. The text deals with the primates on a species-by-species basis and for each species provides information on geographical distribution, population parameters, habitat conditions, factors reported to affect wild populations, and conservation action under way. The profile for each species is accompanied by a distribution map and (where data exist) by a table summarizing population parameters. The distribution maps, which generally indicate *maximum possible* areas of present occurrence, have been carefully prepared and provide reasonably accurate guidelines. In this and other respects, Wolfheim made a concerted effort to ensure accuracy by incorporating results from questionnaires circulated to 226 field workers.

The reference value of *Primates of the World* has been enhanced in a number of ways. Most particularly, it is commendable that the author took pains to use a broadly comprehensible and acceptable scheme of classification; only a conservative scheme, such as the one adopted here, is likely to be readily understandable to the many different groups of people who are likely to use the book. Secondly, the species profiles are followed by a 20-page discussion that identifies a number of general principles, and by seven summary tables (one as an appendix) listing quantitative information across species. Two especially useful tables are one listing "major determinants of population status" (including an estimate of the maximal geographical range for each species in square kilometres) and another relating scores for these determinants to overall conservation status. The quantitative data provided have not been analysed by Wolfheim herself, but should provide at least a provisional basis for mathematical modelling in primate conservation programmes.

However the book does have its limitations. Perhaps unavoidably, it is already quite out of date, coverage of the literature

effectively ending at 1978 (all the references for 1979 and 1980 seem to stem from two edited works). Further, there are no descriptions or illustrations of individual species; hence anyone not already familiar with living primates will need a back-up reference source. It is also a pity that Table 190 gives body weights for only just over half of the primate species, and even then cites maximal body weights largely derived from captive animals. Finally, some of the information (for instance on primate importation and on the associated

legislation) is specifically relevant to the United States only, rather than being genuinely international.

But, overall, Jaclyn Wolfheim has done an excellent job. With one-third of the present total of about 150 primate species already listed as endangered, rare or threatened by the International Union for the Conservation of Nature, a source-book such as this was urgently needed. □

R.D. Martin is Professor of Physical Anthropology at University College London.

A view of life

A.J. Cain

A Synoptic Classification of Living Organisms.

Edited by R.S.K. Barnes.

Blackwell Scientific/Sinauer: 1984. Pp. 273. Pbk £7.50, \$11.50.

DR BARNES has organized a most useful little book, giving a quick view of the diversity of living things, and designed as "the sort of dictionary/mini-encyclopaedia of organismal classification and diversity that all professional, amateur and student biologists could have on their desks". As he remarks, "student biologists are taught less and less of the diversity of life". It is hoped that this book will do something to remedy the defect; yet since it goes only to orders, and there is a single illustration for each phylum, it can do so mainly by sending its readers to more widely-illustrated or detailed texts. As such, it will be valuable.

The classification is a consensus, and avoids some of the wild splitting of recent texts. Viruses are excluded as not coming within the definition of living things, and

only groups with *some* living representatives are admitted — for example, the Merostomata are in, because of the horseshoe crabs, but the trilobites are not; the Chelonia, Rhynchocephalia, Squamata and Crocodilia are in but the various dinosaurs, ichthyosaurs and plesiosaurs are not. Perhaps a later edition can include them.

The Introduction is so brief as to be misleading in one particular: "Taxonomic categories of whatever level are arbitrary, man-made distinctions imposed on a continuum of natural variation" is a dangerous half-truth. It is *not* open to anyone to include human beings, *Drosophila* and snails in a single group. As G.G. Simpson has stressed, taxonomic categories are arbitrary by exclusion, not arbitrary by inclusion. And there isn't a continuum — indeed the definition of phylum status provided in the book is based on gaps.

No one will be entirely satisfied by the classification given here, but the five collaborators have done a good job. May I assist by pointing out that the Marsupialia have been divided into several orders for some time now? □

A.J. Cain is Derby Professor of Zoology at the University of Liverpool.



Tadarida pumila, a molossid bat, drawn by Jonathan Kingdon. The illustration is reproduced from the new paperback edition of Vol. IIA (*Insectivores and Bats*) of Kingdon's magnificent series of books, *East African Mammals: An Atlas of Evolution in Africa*, originally published by Academic Press. Volumes I, IIA and IIB are now available in large-format paperback, published by The University of Chicago Press. Price is \$25, £23 for each volume.

Feel for vertebrates

D.R. Newth

Longman Illustrated Animal Encyclopedia.

Edited by Philip Whitfield.

Longman: 1984. Pp.260. £25. In the United States the Macmillan Illustrated Animal Encyclopedia (Macmillan, \$35).

DO NOT be deceived by the title's implied claim that this book surveys the whole animal kingdom, and still less by the dust-cover which calls it a "visual who's who of all the world's creatures". Instead, more modestly, it treats of some 2,000 species of living vertebrate animal. Each is granted a reproduction, in colour, of a painting and a short (80–300-word) non-technical description. The aim has been to include at least one species from each tetrapod family and at least one from each order of the fishes. Some higher taxa are briefly described and mammals, birds, reptiles, amphibians and fishes are each introduced by a short general account.

The pictures are certainly both vivid and decorative, but they are not always

sufficiently detailed or accurate to match good colour photographs (although, of course, good photographs would not always be easy to obtain). Since they are rationed at one to each species there is no place for larval or juvenile forms, and where sexual dimorphism is marked the more flamboyant sex is chosen for show. The textual material is necessarily basic, but has lacked critical revision to eliminate minor errors and other lapses from grace. Of the latter, the heading given to the section on reptiles, which boldly proclaims them to be "survivors from a prehistoric age", seems typical. It is true, but true also of every other class of living animal.

Its defects apart, it is unlikely that this book will be of value to students of vertebrate zoology. On the other hand it may well attract and instruct non-scientists and give them a feel for the range of size, shape, coloration and lifestyle of the vertebrates and some idea of their relationships. This, presumably, was the intention of the publishers, but it is still difficult to imagine whose coffee table the book is designed to grace. □

D.R. Newth was formerly Regius Professor of Zoology at the University of Glasgow.

Down under standard

G.B. Corbet

The Complete Book of Australian Mammals.

Edited by Ronald Strahan.

Angus & Robertson: 1984. Pp.529. £25.

THE past 20 years have witnessed a revolution in the application of high-quality colour printing to natural history publishing. In that same period the production of authoritative syntheses of biological data on major taxonomic groups and faunal regions has achieved increasingly high standards. But with some exceptions, the economics of publishing have usually prevented these advances from coming together. Writers and editors of scholarly works are commonly frustrated by the impracticality of including colour illustrations, even when these would enormously enhance the information content, whilst popular works with adequate, and often superb, colour illustration rarely carry a text that meets the standards required of a scientific source book.

The exceptions have mostly dealt with subjects such as molluscs and butterflies, where the ease of photographing preserved specimens and the popular interest have combined to make good illustrated handbooks feasible. The elusiveness of most mammals makes them a much more difficult subject for such treatment, and so *The Complete Book of Australian Mammals* is something of a pioneer. It is based upon a

National Photographic Index of Australian Wildlife established by the Australian Museum, Sydney, and includes colour photographs of living animals of all but 22 of the 273 species included. Considering the rarity and elusiveness of many Australian mammals, marsupials and placentals, this is quite an achievement.

Although the convergence and parallelism shown by the Australian marsupials in relation to placental mammals elsewhere are a standard ingredient of evolutionary teaching, the illustration of almost all the 129 species in one work enables this phenomenon to be appreciated and indeed studied in a way that has not hitherto been possible.

The text is the work of 107 different authors with first-hand research experience, and is up-to-date and authoritative, summarizing habitat, ecology, reproduction and behaviour. The reference value is greatly enhanced by a clear distribution map for each species. Although facts are not individually documented with regard to source, some bibliographical references are given for each species, providing at least an entry into the literature.

While it falls short of being a really comprehensive illustrated handbook — it does not, for example, cover critical identification requiring internal characters — this book nevertheless sets a standard that has not yet been matched in areas such as Europe and North America where the mammal fauna is much better known. □

G.B. Corbet is in the Department of Central Services at the British Museum (Natural History), London.

Everyone's stunning animals

Carolyn M. Crockett

The Encyclopedia of Mammals.

Edited by David Macdonald.

George Allen & Unwin/Facts on File: 1984. Pp. 895. In Britain published in two volumes, £25 each. In the United States as a single volume, \$45.

HERE is a volume whose illustrations and photographs are a pleasure to peruse. What makes it an especially worthwhile purchase, however, is the authoritative and well-written text. David Macdonald has orchestrated a lively collection of articles, assembling contributions from 180 experts from around the world; the result will appeal to anyone interested in animals — the lay public, students and their teachers, and professional zoologists.

The book describes the appearance, diet, social behaviour, anatomical characteristics and peculiarities of all mammal groups. For some, illustrations and descriptions of extinct ancestors are also included. The entries are all short, ranging from a small box on a specific topic to several pages of description of a mammal group, usually at the family or subfamily level. This makes ideal reading with morning coffee or afternoon tea, since the individual passages hang together and can be read in just a few minutes.

The book begins with a dozen or so pages describing mammals in general, summarizing their evolution, characteristics and taxonomy. The remainder is divided into accounts of each order and concludes with a bibliography, a glossary of nearly 500 terms that might not be familiar to the lay reader, and an index. For most orders, a complete listing and short description of each species is given in a section of the main text. A few orders, such as the Rodentia, Chiroptera and Marsupialia, are described down to the genus or tribe level, while a complete listing of species is presented in an appendix.

To give an indication of the richness of this volume, let me summarize the section on the Capuchin-like monkeys (Cebidae). It begins with a box containing a distribution map and a silhouette scale-drawing comparing the size of a man with the largest and the smallest members of this monkey group. The text includes a general description of the Cebids, their adaptations (such as prehensile tails for the larger species) and their social organization. A beautiful colour drawing (one of many excellent ones by Priscilla Barrett) accurately depicts nine species in various postures; by showing different — yet typical — behaviours, this and the other plates provide more information than do most field guides. Next comes a more traditional description of each species, including common name,

scientific name, pelage, distribution and so on, and finally four "topical" accounts of one to two pages each — here, exploration and play in Squirrel monkeys; why Night monkeys are nocturnal; the family life of Titi monkeys; and the energetic constraints imposed on Howler monkeys by their relatively unnutritious diet. Photographs are interspersed throughout.

As an amateur photographer and illustrator, I feel compelled to emphasize the sheer beauty and artistry of the layout and illustrations. Many of the photographs are stunning, portraying the very essence of an animal's life. One of the most dramatic depicts a herd of wildebeest plummeting into a swirling river.

There are really only two drawbacks to the book, both of them minor. First, no specific references are given (though each entry is initialled and in many cases the author's publications could be consulted). Second, while several passages, especially the "topical" ones, discuss controversial matters, in some instances no indication is given that this is the case; the problem then arises of preliminary conclusions being perpetuated as truths. But it is yet another merit of this splendid work that it corrects a far greater number of myths than it creates.

Carolyn M. Crockett is a Research Associate in the Departments of Anthropology and Psychology at the University of Washington, Seattle, and at the Smithsonian Institution, Washington, DC.

Mammoth appeal

Brian Bertram

The Mammals of the Southern African Subregion.

By Reay H.N. Smithers.

University of Pretoria: 1983. Pp.736.

Distributed by Struik Book Distributors, PO Box 1144, Cape Town 8000, South Africa. £53, \$70.

PRODUCING this major reference work on southern African mammals is described in the foreword as "a mammoth undertaking". It is a very apt description, for mammoths are also enormous, still too little known, fascinating in detail and just a bit dated in appearance.

Reay Smithers has managed during a relatively short gestation period of only about four years to assemble and present a vast amount of information. The three-quarters of a million words cover 340 species of mammal, and there are tables, illustrations and distribution maps as well. It is formidable — and remarkable that the book is still readable too.

The "Southern African Subregion" includes South Africa, Namibia, Botswana, Zimbabwe south of the Zambezi River and the Prince Edward Islands (situated 900 miles south of the con-

tinents), as well as the adjacent oceans. The latter provide an excellent excuse for a useful section on marine mammals — the whale and seal orders. Each species entry is divided between several subheadings: colloquial name; taxonomic notes (covering subspecies and disagreements over classification); description (including, at last, valuable tables giving data on body sizes and weights); distribution; habitat; habits (an inadequate term for the wealth of behaviour and ecology described); food reproduction; and skull (with dental formula).

The possible disadvantage of a standard format such as this is that sparse or trivial data might be included for some species (because there is a heading for them) and major topics omitted for better documented species. Fortunately Smithers has not felt constrained by the format: he includes introductory and general sections, as well as topics of particular interest (such as fur seal harvesting, and the multimammate mouse and human diseases), and he makes quite clear where adequate information is lacking. The gaps are fast being filled in, and what would have been only a skeleton of a mammoth 20 years ago is becoming beautifully padded out, thanks to the high quality of field work carried out and published over the past two decades or so.

The book's publication in South Africa in 1983, and its recent availability worldwide, make clear the extent of the takeover by southern Africa as the centre for exciting work on wild African mammals. Fifteen years ago, East Africa with its wildlife research groups particularly in Serengeti, Gombe, Nairobi, Tsavo and Mweya, was leading Africa if not the world in large-vertebrate field studies. For many reasons — political, financial and personal — the East African centres have become reduced. At the same time, the value of such research institutes has been amply demonstrated by the success of the Mammal Research Institute in Pretoria, which has both sired this excellent volume and provided much of the field data on which it is so soundly based. Fortunately, the whole of Africa, and indeed the whole mammal world will benefit. □

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● One of the best-selling publications issued by the British Library's Science Reference Library in London, *Guide to Government Department and Other Libraries*, has recently appeared in a new edition (the twenty-sixth). The entries cover not only sources of information within Government departments, but a selection of other specialist libraries and include many dealing with science and technology.

The *Guide* is available from: External Relations and Liaison Section, Science Reference Library, 25 Southampton Buildings, London WC2A 1AW, UK. Price is £10 (£11.28 for overseas orders); cheques should be made payable to The British Library.

Up in the air over the nation

John Andrews

A Field Guide to the Birds of the USSR.

By V.E. Flint *et al.*

Translated by Natalia Bourso-Leland.

Princeton University Press: 1984. Pp.353. \$65, £54.20.

The Birds of China.

By Rodolphe Meyer de Schauensee.

Smithsonian Institution Press/Oxford University Press: 1984. Pp.602. Hbk \$45, £35; pbk \$29.95.

A Pictorial Guide to the Birds of the Indian Subcontinent.

By Salim Ali and S. Dillon Ripley.

Oxford University Press: 1984. Pp.177. £22.50.

Field Guide to the Birds of North America.

Edited by Shirley Scott *et al.*

National Geographic Society/ David and Charles: 1984. Pp.464. Pbk \$13.98, £8.95.

IT IS rare that readers of ornithological reviews are vouchsafed gratuitous political comment, but the production in one year of English-language field guides for the super-powers and the largest of the non-aligned nations can hardly pass unremarked. The problem is to know what this signifies — capitalist exploitation of new commercial opportunities or a shared concern for the natural world? Whatever the explanation, we can value each book for its own sake because all four of them make a worthwhile contribution to accessible knowledge.

A Field Guide to the Birds of the USSR was originally published in Russian in 1968 but soon dropped out of print and became, for practical purposes, unavailable in the West. This English-language edition has been updated by the senior author, Dr Vladimir Flint, in collaboration with advisers from the Massachusetts Audubon Society. A new introduction provides the Western reader with a very generalized account of the major habitat divisions of the USSR and their characteristic bird communities. The 750 individual species-descriptions comprise a summary of field marks, drawing attention to features aiding the separation of similar species, plus information on habitats, behaviour, nesting and distribution, with maps. The colour plates are good, and publisher and printer deserve credit for the standard of colour correction, which, when badly done, often mars good origination.

Though smaller than the USSR, China contains nearly 1,200 bird species, straddling as it does the palaearctic and oriental faunal regions. *The Birds of China* provides field descriptions of all adults, but lacks the information on habits so helpful to field identification. Distribution is

described in terms of provinces, which are mapped on the endpapers. The plates cover less than half the species occurring, and are based on museum specimens which are frankly described as being "of varying ages". Not unwisely, the illustrators — John Henry Dick, John Gwynne and H. Wayne Trimm — have largely adopted a somewhat cartoonish style, with little attempt to show precise plumage detail or jizz. The shortcomings of the work largely reflect the lack of information, and when one realizes that the artists and the author are based in the United States one must applaud the achievement rather than criticize. Sadly, there is as yet no edition published in China.

A Pictorial Guide to the Birds of the Indian Subcontinent shares many species with the previous two books, and with them serves to create a mutually supportive trilogy of guides. It is a useful work in its own right, however, the strongest point being the illustrations by John Henry Dick which cover all species, including females and some juveniles, plus flight illustrations of raptors, some waterfowl and seabirds. In comparison the text is disappointing, comprising little more than the briefest statement of habitat preference and distribution, derived from and cross-referenced to Ali and Ripley's ten-volume *Handbook of the Birds of India and Pakistan* published between 1969 and 1974.

All three of the books discussed above owe their present incarnations to American initiative or co-operation, and the pre-eminence of the United States in the compilation of ornithological reference works is underlined by the *Field Guide to Birds of North America*. This book is the fruit of work by four consultants, nearly forty editorial and production staff and thirteen main illustrators, but what sounds like a recipe for unmitigated disaster has produced a guide of remarkable scope and high standards and utility. The colour illustrations cover most plumage variations, enabling one to compare, say, juvenile crowned and yellow-crowned night herons or flying juveniles of the light and dark phases of skuas — sorry, jaegers! With, on average, four species per plate, there is adequate room on the facing page for helpful descriptive text and distribution maps of varying scale, enabling more precise definition of more localized species. In practical terms, the *Guide* has the virtue of covering the whole of North America in a single book, unlike the otherwise excellent Peterson volumes. It should also find a market on the eastern side of the Atlantic, for its usefulness in relation to those species we normally share as well as for the vagrants which regularly test European ornithologists each autumn. □

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Fish: relations and compositions

D. H. Cushing

Fishes of the World, 2nd Edn.

By Joseph S. Nelson.

Wiley: 1984. Pp.523. £52, \$44.95.

Marine Fishes.

Edited by V.P. Bykov.

Balkema: 1984. Pp.322. Dfl. 50, £11.50.

PROFESSOR Nelson's authoritative text, the updated edition of a book first published in 1976, describes the classification of fishes down to families and it lists the genera. There is an illuminating discussion on the nature of systematics, cladistic or synthetic, but no hint of the recent flurries in this area. Each order is defined in anatomical terms, as is each sub-order and family; in each description there are indications of ecological status. The drawings are very clear and simple, and are linked quite exactly with the anatomical description, while on the end-papers there is a helpful diagrammatic presentation of the hierarchy of the higher categories of fishes. Links to the fossil groups are well established and, indeed, the fossil genera are listed in the description of each order. There are 45 maps showing the distribution of a class or of families throughout the world.

The volume edited by Dr Bykov is superficially similar to Professor Nelson's, in that the fish species described from Russian catches are grouped by families within two super-orders, Selachioidei and Teleostei. There the similarity ends for this classification is a convenience, not an end in

itself, and the real point of the book is to list the chemical composition by species, region and season and by parts of the body. In certain cases methods of processing are given in some detail. There is no attempt to describe each species for the illustrative drawing is probably considered sufficient; such drawings are well executed and can be matched with those of Professor Nelson. So far as I can see the species are correctly named, and while the book is of somewhat specialist interest there are useful snippets of information scattered throughout the text.

As works of reference the two volumes serve different readerships. That edited by Dr Bykov will be used on far-ranging factory ships and in processing plants where an indication of processing quality of each species caught would be of considerable value. (The book, incidentally, also indicates how far the Russian fleet works away from its home waters, the Barents Sea, the Baltic and the north-west Pacific; in the Barents Sea, species and their chemical composition would be well known). There are two readerships for Professor Nelson's text. First, museum systematists will be glad to have the classification set out so clearly for those groups in which they are not experts. Secondly, fishery biologists whose trade is population dynamics should profit from the arrangement of orders; the volume should be in every library where fisheries are studied. For example I have used it recently to track certain genera unfamiliar to me in a study of the marine resources of the western Indian Ocean. □

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